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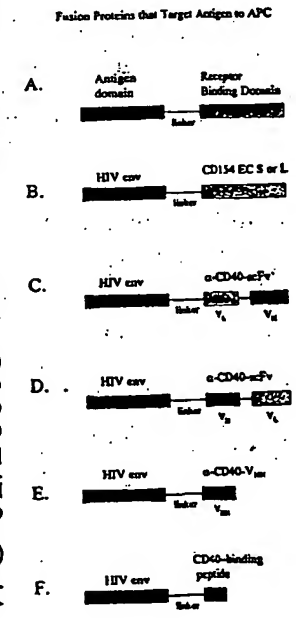
(54) Title: DNA VACCINES ENCODING ANTIGEN LINKED TO A DOMAIN THAT BINDS CD40



(57) Abstract: Vaccines that target one or more antigens to a cell surface receptor improve the antigen-specific humoral and cellular immune response. Antigen(s) linked to a domain that binds to a cell surface receptor are internalized, carrying antigen(s) into an intracellular compartment where the antigen(s) are digested into peptides and loaded onto MHC molecules. T cells specific for the peptide antigens are activated, leading to an enhanced immune response. The vaccine may comprise antigen(s) linked to a domain that binds at least one receptor or a DNA plasmid encoding antigen(s) linked to a domain that binds at least one receptor. A preferred embodiment of the invention targets HIV-1 env antigen to the CD40 receptor, resulting in delivery of antigen to CD40 positive cells, and selective activation of the CD40 receptor on cells presenting HIV-1 env antigens to T cells.

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TITLE: DNA VACCINES ENCODING ANTIGEN LINKED TO A DOMAIN THAT BINDS CD40

CROSS REFERENCE TO RELATED APPLICATIONS

This application is entitled to the benefit of Provisional Patent Application Ser. # 60/159,690, filed 1999 October 14.

TECHNICAL FIELD:

This invention relates to DNA vaccines, specifically to improved DNA vaccines that induce strong antigen-specific humoral and cellular immune responses.

BACKGROUND ART:

DNA immunization, the inoculation of plasmid DNA encoding a microbial or tumor antigen, is a recent addition to vaccine technology (Donnelly J.J. et al, Ann. Rev. Immunol. 15: 617-648, 1997; Letvin N. L., Science 280: 1875-1879, 1998). Both cellular and humoral immune responses occur after DNA vaccination, and protective immunity against microbial challenge is sometimes induced in experimental animals (Ulmer J.B. et al, Vaccine 12: 1541-1544, 1994; Yokoyama M. et al, J. Virol. 69: 2684-2688, 1995; Xiang Z.Q. et al, Virology 199: 132-140, 1994; Sedegah M. et al, Proc. Natl. Acad. Sci. USA 91: 9866-9870, 1994; Montgomery D.L. et al, DNA Cell Biol. 12: 777-783, 1993). T cell responses, including CD8+ cytotoxic T lymphocyte (CTL) and CD4+ T helper cells, can be stimulated by DNA vaccination in response to antigenic peptides presented by class I and class II MHC molecules (Whitton J.L. et al, Vaccine 17: 1612-1619, 1999).

Endogenous protein synthesis allows presentation of foreign antigenic peptides by MHC class I, whereas uptake of soluble protein by APC is required for presentation of peptides by MHC class II. Both arms of the immune response can therefore be induced after DNA vaccination, but the pathways for antigen processing and presentation are distinct for peptides presented by MHC class I or MHC class II. This conclusion is derived from experiments using DNA encoding ubiquitinated protein that is rapidly targeted to intracellular degradation by proteosomes. Ubiquitinated antigen that was degraded so rapidly that intact protein could not leave the cell led to enhanced production of CTL *in vivo*, but completely eliminated antibody production (Rodriguez F. et al, J. Virol. 71: 8497-8503, 1997; Wu Y. and Kipps T.J., J. Immunol. 159: 6037-6043, 1997). Thus a major limitation of DNA vaccines is their inability to induce strong and sustained humoral immune responses. Strategies for optimization of the cellular immune response to DNA vaccines that do not reduce humoral immune responses are needed.

DNA vaccines for HIV-1 have been tested in animal models and found to induce an immune response that provides protection against challenge only when the virulence of the viral isolate is low. In benign challenge models, chimpanzees were protected from live virus exposure by vaccination with plasmid DNA or by subunit antigens or peptides (Boyer J.D. et al, Nat. Med. 3:526-532, 1997; Kennedy R.C., Nat. Med. 3: 501-502, 1997). However, when highly virulent SIV was tested in rhesus macaques, DNA vaccination was not protective and could only achieve a reduction in virus load even when multiple doses of DNA were inoculated through multiple routes (Lu S. et al, J. Virol. 70: 3978-3991, 1996). Therefore, enhancing the immune response to DNA immunization is an important goal of current AIDS vaccine research. Enhancing the immune response to other DNA vaccines is also desirable in order to provide protection when infected with highly virulent organisms or with a high infectious dose, and to provide long lasting protection. Enhancing the immune response to DNA vaccines encoding tumor antigens is also important for maximizing the anti-tumor response.

One strategy that has been tested is to prime with a DNA vaccine followed by boosting with protein antigen. However, this approach requires construction of multiple vaccines for the same infection or disease, and depends upon multiple injections given in a precise order. It would be desirable to induce protective immunity without needing

multiple forms of a vaccine, and without requiring alternating injections of DNA and protein.

Chemical and genetic approaches to enhance the immune response to DNA vaccines have been studied. Chemical adjuvants with some activity include monophosphoryl lipid A (Sasaki S. et al, Infect. Immun. 65: 3520-3528, 1997), saponin QS-21 (Sasaki S et al, J. Virol. 72: 4931-4939, 1998), mannan-coated liposomes (Toda S et al, Immunology 92: 111-117, 1997), and the aminopeptidase inhibitor ubenimex (Sasaki S et al, Clin. Exp. Immunol. 11: 30-36, 1998). Each of these adjuvants modestly enhanced both antibody titers and CTL activity after DNA vaccination in mice. Although the mechanism of action of chemical adjuvants is not fully elucidated, they seem to work by induction of cytokines that amplify responses, by recruitment of macrophages and other lymphoid cells at sites of DNA administration, or by facilitating entry of DNA into host cells (Sasaki S. et al, Anticancer Research 18: 3907-3916, 1998). Several genetic approaches to enhancing responses to DNA vaccines have been tested, including administration of a gene encoding a cytokine (IL2, IL12, GM-CSF, TCA3, MIP-1α) (Chow Y.-H. et al, J. Virol. 71: 169-178,1997; Hwee Lee A. et al, Vaccine 17: 473-479, 1998; Tsuji T. et al, Immunol.158: 4008-4014, 1997; Rodriguez D. et al, Gen.Virol.80: 217-223, 1999; Tsuji T. et al, Immunology 90: 1-6, 1997; Lu Y. et al, Clin. Exp. Immunol. 115: 335-341,1999) or a costimulatory adhesion receptor (CD86, CD58, CD54) (Tsuji T. et al, Eur. J. Immunol. 27: 782-787, 1997; Kim J.J. et al, J. Clin. Invest. 103: 869-877, 1999; Iwasaki A. et al, J. Immunol. 158: 4591-4601, 1997). Each of these cytokine and adhesion receptor genes increased immune responses to DNA vaccination, with some treatments enhancing CTL generation only, and some enhancing both CTL and antibody production. However, the levels of enhancement of the immune response to DNA vaccination obtained from these approaches are modest and not sustained, so it is important to find additional ways to enhance the immune response to DNA vaccines.

The CD40 receptor must be activated for an effective cellular or humoral immune response after exposure to antigen (Grewal I.S., and Flavell R.A., Annu. Rev. Immunol 16: 111-135, 1998). This conclusion is derived from multiple findings, including the phenotype of patients with hyper IgM (HIGM) syndrome that results from CD154

genetic defects (Aruffo A. et al, Cell 72: 291-300,1993; Fuleihan R. et al, Proc. Natl. Acad. Sci. USA 90: 2170-2173,1993; Korthauer U. et al, Nature 361: 539-541,1993), the phenotype of mice with CD40 or CD154 gene disruption (Grewal I.S. et al, Science 273: 1864-1867,1996; Kawabe T. et al, Immunity 1: 167-178,1994; Renshaw B. et al, J. Exp. Med. 180: 1889-1900,1994; Xu J. et al, Immunity 1: 423-431, 1994), and the effects of actively blocking CD40 *in vivo* using inhibitory antibodies to CD154 (Durie F.H. et al, Science 261: 1328-1330,1993; Foy T.M. et al, J. Exp. Med. 178: 1567-1575, 1993; Foy T.M. et al, J. Exp. Med. 180: 157-163,1994; Durie F.H. et al, J. Clin. Invest. 94: 1333-1338, 1994; Gerritsse K. et al, Proc. Nat. Acad. Sci. USA 93: 2499-2504, 1996). CD40 is expressed in several cell lineages, including B cells, dendritic cells, monocytes, epithelial cells, and endothelial cells. CD40 transmits signals for each of these cell types that regulates activation and differentiation (Hollenbaugh D. et al, EMBO J. 11: 4313-4321,1992; Kiener P.A. et al, J. Immunol. 155: 4917-4925,1995; Cella M. et al, J. Exp. Med. 184: 747-752,1996; Galy A.H., and Spits H., J. Immunol. 152: 775-782,1992; Clark E.A., and Ledbetter J.A., Proc. Natl. Acad. Sci. USA 83: 4494-4498, 1986). CD40 is activated by crosslinking during cell to cell contact with cells expressing CD40 ligand (CD154), primarily T cells. While soluble forms of CD154 can stimulate CD40, no attempts have been made to use or modify soluble CD154 to promote immune responses to antigens.

CD40 signals to B cells are required for isotype switching and affinity maturation through somatic mutation (Rousset F. et al, J. Exp. Med. 173: 705-710, 1991). In the absence of CD40 signals, germinal centers, the specialized sites of B cell maturation, are not formed, and B cells are unable to differentiate into IgG producing plasma cells (Foy T.M. et al, J. Exp. Med. 180: 157-163, 1994). Patients with HIGM syndrome are not able to form germinal centers or produce IgG antibodies after antigen challenge, and the same phenotype is seen in knockout mice where CD40 or CD154 is not expressed. The CD40 signal has been shown *in vitro* to promote survival of surface Ig-activated B cells, and to interact with signals from cytokines to induce immunoglobulin isotype switching to IgG, IgA, and IgE production (Holder M.J. et al, Eur. J. Immunol 23: 2368-2371,1993; Jabara H.H. et al, J. Exp. Med. 177: 925-935,1990; Grabstein K.H. et al, J. Immunol. 150: 3141-3147, 1993). In addition, HIGM syndrome patients and CD154 knockout mice have impaired lymphocyte proliferation in response to diphtheria toxoid,

tetanus, and *Candida*, showing that the CD40 signal is required for T cell priming to protein antigens (Grewal I.S., and Flavell R.A., Annu. Rev. Immunol 16: 111-135, 1998; Toes R.E.M. et al, Sem. Immun. 10: 443-448,1998; Grewal I.S. et al, Nature 378: 617-620,1995; Ameratunga R. et al, J. Pediatr. 131: 147-150,1997; Subauste C.S. et al, J. Immunol. 162: 6690-6700, 1999). Expression of CD154 *in vivo* to enhance immune responses utilized only the cell surface form of the molecule and resulted in significant toxicity in experimental animals, including induction of lethal autoimmune disease and T cell malignancies (Roskrow M.A et al, Leukemia Research 23: 549-557, 1999; Brown M.P. et al, Nature Medicine 4: 1253-1260, 1998).

In neonates, insufficient stimulation of CD40 due to low levels of expression of CD154 by activated T cells has been identified as a factor in the inability of infants to produce IgG antibodies towards bacterial antigens (Nonoyama S. et al, J. Clin. Invest. 95: 66-75, 1995; Fuleihan R. et al, Eur. J. Immunol. 24: 1925-1928, 1994; Brugnoni D. et al, Eur. J. Immunol. 24: 1919-1924, 1994). This suggests that CD40 signals are not ubiquitous and that highly restricted expression of CD154 may limit the extent of CD40 signaling and thus the magnitude and quality of an immune response. Direct evidence in support of this idea comes from a recent study where a modest increase (1.1-2 fold) in expression of cell surface CD154 in the thymus of mice resulted in a > 10 fold increase in the antigen-specific antibody response (Prez-Melgosa M. et al, J. Immunol. 163: 1123-1127, 1999). Some evidence suggests that CD40 stimulation may be deficient in HIV-1 infected individuals, since HIV gp120 suppressed the expression of CD154 by activated T cells *in vitro*, and production of IL12 is defective in HIV-1 positive individuals (Chirmule N. et al, J. Immunol. 155: 917-924, 1995; Taoufik Y. et al, Blood 89: 2842-2848, 1997; Yoo J. et al, J. Immunol. 157: 1313-1320, 1996; Ito M. et al, AIDS Res. Hum. Retroviruses 14: 845-849, 1998; Benyoucef S. et al, J. Med. Virol. 55: 209-214, 1998). In addition, CD40 stimulation of dendritic cells infected with HIV-1 was found to suppress virus replication, suggesting that transmission of HIV-1 from infected dendritic cells during antigen presentation could be blocked by CD40 signals (McDyer J.F. et al, J. Immunol. 162: 3711-3717, 1999). However, a method for stimulation of CD40 on cells actively presenting antigen to T cells while avoiding toxicity from unregulated CD40 stimulation is needed.

CD40 signals to dendritic cells or B cells causes their differentiation from an antigen uptake function to an antigen processing and presentation function (Sallusto D. et al, J. Exp. Med. 182: 389-400, 1995; Cella M. et al, J. Exp. Med. 184: 747-752, 1996; Faassen A.E. et al, Eur. J. Immunol. 25: 3249-3255, 1995). This shift is accompanied by reduction of the MHC class II intracellular compartment, increased expression of MHC class II on the cell surface, secretion of the Th1 regulatory cytokine IL12 and increased expression of CD86 and CD80. After CD40 activation, dendritic cells and B cells are able to more efficiently present antigen and give a critical costimulatory signal through CD28. The production of IL12 leads to enhanced secretion of IFNγ by T cells and suppression of Th2 cytokine production. The CD40 signal is therefore an important mediator of Th1 cellular immunity and CTL induction. However, selective stimulation of CD40 during antigen presentation is needed to enhance immune responses to vaccination.

In addition to B cells and dendritic cells, CD40 is functionally active on other APC's such as monocytes, where CD40 signals prevent cell death from apoptosis and induce expression of adhesion molecules and production of inflammatory cytokines TNFα and IL8 (Kiener P.A. et al, J. Immunol. 155: 4917-4925, 1995). CD40 has also been reported to be expressed and functionally active on thymic epithelial cells (Galy A.H., and Spits H., J. Immunol. 152: 775-782, 1992) and on many kinds of tumor cells, including carcinomas, melanomas, and lymphomas (Ledbetter J.A. et al, In Leucocyte Typing III: White Cell Differentiation Antigens p. 432-435, 1987; Oxford University Press, Oxford, U.K.; Paulie S. et al, Cancer Immunol. Immunother. 20: 23-28, 1985). In contrast to most normal cells where the CD40 signal enhances survival, in many malignant cells CD40 actively promotes death by apoptosis. Therefore CD40 is functionally active in all cell types that express the receptor, and CD40 signals are central to fundamental processes of survival and differentiation. Because of the widespread expression of functional CD40, localized stimulation of CD40 positive cells that present specific antigen to T cells is desirable so that only APC involved in the specific immune response are activated.

Studies in CD154 knockout mice have confirmed the importance of CD40 activation for the antigen specific priming of T cells. CD154 deficient mice have an

enhanced susceptibility to *Leishmania major* and *Toxoplasma gondii* infection, consistent with a central role for CD40 in cellular immunity (Subauste C.S. et al, J. Immunol. 162: 6690-6700, 1999; Campbell K.A. et al, Immunity 4: 283-289, 1996). CTL generation after viral infection in CD154 deficient mice is markedly blunted, and induction of experimental allergic encephalomyelitis (EAE) in response to myelin basic protein does not occur (Grewal I.S. et al, Science 273: 1864-1867, 1996; Grewal I.S. et al, 378: 617-620, 1995). The defect in T cell priming in these models appears to be due to an inability of APC to provide costimulatory signals to T cells (Grewal I.S. et al, Science 273: 1864-1867, 1996; Yang Y. and Wilson J.M., Science 273: 1862-1867, 1996).

Inhibition of CD40 *in vivo* has been studied in mice using a mAb, MR1, that binds and blocks the CD40 ligand, CD154 (Durie F.H. et al, Science 261: 1328-1330, 1993; Foy T.M. et al, J. Exp. Med. 178: 1567-1575, 1993; Foy T.M. et al, J. Exp. Med. 180: 157-163, 1994; Durie F.H. et al, J. Clin. Invest. 94: 1333-1338, 1994; Gerritsse K. et al, Proc. Nat. Acad. Sci. USA 93: 2499-2504, 1996). These experiments demonstrated that anti-CD154 prevents the induction of autoimmune diseases, including EAE after immunization with myelin basic protein, oophritis after immunization with zona pelucida antigen (ZP3), and spontaneous disease in lupus prone mice (Griggs N.D. et al, J. Exp. Med. 183: 801-807, 1996; Daikh D.I. et al, J. Immunol. 159: 3104-3108, 1997). Anti-CD154 was also effective in preventing both chronic and acute graft versus host (GVH) disease and in preventing rejection of heart allografts after transplantation (Larsen C.P. et al, Nature 381: 434-438, 1996). Thus, CD40 signals are required for T cell responses to antigen, and restriction of the CD40 signal with specific inhibitors is an effective method of limiting T cell priming during an immune response.

The CD40 receptor is therefore a proven target for regulation of antigen specific immunity. While biological inhibitors of CD40 have been studied extensively in mice and in nonhuman primates, there is a need for localized stimulation of CD40 on cells that present antigens to T cells in order to improve the effectiveness of vaccines.

Gp160, the product of the HIV-1 env gene, is cleaved in the Golgi complex into gp120 and gp41 proteins that remain associated through noncovalent interactions. Most

neutralizing epitopes of the virus are located on gp120 and gp41, and are expressed by the intact env complex that has been shown to be a trimer (Kwong P.D. et al, Nature 393: 648-659, 1998). Monomeric gp120 can be released from the complex and expose immunodominant epitopes that are non-neutralizing and are located on the internal face of gp120 in the intact trimeric complex (Wyatt R. et al, Nature 393: 705-711, 1998; Broder C.C. et al, PNAS USA 91: 11699-11703, 1994). Thus, stabilization of the env complex is needed for an HIV-1 vaccine in order to preserve conformational epitopes important for neutralization and to mask immunodominant epitopes that are not relevant for neutralization of the env complex.

One attempt to produce a stable, properly folded gp120-gp41 complex was made by altering the cleavage site in gp160 between the gp120 and gp41 domains (Earl P.L. et al, J. Virol. 68: 3015-3026, 1994). By introducing a stop codon before the transmembrane domain of gp41, a soluble molecule composed of gp120 and the extracellular domain of gp41 was produced as a complex that folds properly to bind the CD4 receptor and to express some conformational epitopes. However, this molecule formed dimers and multimers rather than the stable trimers that comprise the native structure of the envelope glycoprotein as revealed in the crystal structure of the gp120 complex.

Three major sites of gp120 have been identified that are involved in cross-neutralization of diverse viral strains (Wyatt R. et al, Nature 393: 705-711, 1998). The V3 domain was found to express linear and conformational epitopes that can be recognized by antibodies that neutralize HIV-1. Although the V3 domain is a variable region, it contains a central portion shared by many HIV-1 isolates, particularly those found in the United States and Europe. The central portion has been called the principle neutralization epitope and is formed from a linear epitope of the amino acid sequence GPGRAF (Broliden P.A. et al, Proc. Natl. Acad. Sci. USA 89: 461-465, 1992; Broliden P.A. et al, Immunol. 73: 371-376, 1991; Javaherian K. et al, Science 250: 1590-1593, 1990; Javaherian K. et al, Proc. Natl. Acad. Sci. USA 86: 6768-6772, 1989). Conformational epitopes of the V3 loop have also been identified that can be recognized by antibodies that are more broadly neutralizing.

The CD4 binding domain of gp120 is another neutralization site for antibodies directed to HIV-1 env. This domain is a nonlinear, conformational site that depends upon proper folding of gp120 (Kang C.-Y. et al, Proc. Natl. Acad. Sci. USA : 6171-6175, 1991; Lasky L.A. et al, Cell 50: 975-985, 1987). Antibodies can recognize distinct portions of the CD4 binding domain, and may have either type-specific or cross-neutralization properties (Pinter A. et al, AIDS Res. Hum. Retro. 9: 985-996, 1993). Although monomeric gp120 can retain CD4 binding function, a stable trimeric structure of gp120 is thought to be important for masking immunodominant epitopes that are expressed on the internal face of the intact complex (Wyatt R. et al, Nature 393: 705-711, 1998). A third domain of gp120 involved in virus neutralization is exposed upon binding to CD4, and functions to bind the chemokine coreceptor to allow virus entry into the cell (Rizzuto C.D. et al, Science 280: 1949-1953, 1998). Thus a stable trimer of HIV-1 env is needed to present the major cross-neutralization epitopes and to prevent exposure of internal, immunodominant epitopes that do not induce neutralizing antibodies.

CD154 is a TNF-related, type II membrane protein that forms stable trimers (Mazzei G.J. et al, J. Biol. Chem. 270: 7025-7028, 1995). Soluble fusion proteins of human CD154 have been expressed using murine CD8 at the amino terminal side of the CD154 molecule (Hollenbaugh D. et al, EMBO J. 11: 4313-4321, 1992). Single chain Fv (scFv) molecules have also been constructed using heavy and light chain variable regions cloned from the G28-5 hybridoma that produces antibody specific for human CD40 (Ledbetter J.A. et al, Crit. Rev. Immunol.17: 427-435, 1997). Both CD154 and G28-5 scFv fusion proteins retain functional activity as soluble molecules *in vitro*. However, no use of these molecules to improve the effectiveness of vaccines has been found.

DISCLOSURE OF INVENTION

For vaccines to be effective, they must induce both humoral and cellular immune responses. This invention describes improved vaccines that target antigens to cell surface receptors. DNA vaccines are a recent addition to immunization technology. However, further optimization of DNA vaccines is needed to induce long-lasting

protection against tumor antigens, virulent HIV-1 isolates, and other pathogenic microorganisms. Receptor activation and targeting improves the ability of DNA vaccines to generate strong cellular immunity and high titers of neutralizing antibodies. CD40 is a preferred receptor for targeting and activation. DNA vaccines encoding CD40 ligand (CD154) or a single chain Fv (scFv) specific for CD40, fused with DNA encoding portions of the HIV-1 env protein are preferred embodiments of the invention. A molecule comprising the extracellular domain of HIV-1 env gp160 or env gp120 linked to the extracellular domain of CD154 is a stable trimer that improves immune recognition of HIV-1 env cross-neutralization epitopes. After DNA vaccination, the expression of the fusion protein *in vivo* results in both activation of the CD40 receptor and direction of HIV-1 env antigens into the endocytic pathway of CD40 positive antigen presenting cells (APC). Internalization of env antigens after binding the CD40 receptor enhances presentation of peptides by MHC molecules. Activation of the CD40 receptor promotes B cell and APC maturation leading to effective antibody production and generation of CD4+ helper T cell and CD8+ CTL activity. The combination of CD40 activation, stabilization of the HIV-1 gp160 or gp120 env trimer, and enhanced presentation of antigenic peptides by MHC molecules thus improves immune responses to HIV-1 antigens. Protein molecules of the invention can be injected directly into mammals or encoded by DNA vaccines.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1.

Schematic representation of fusion proteins that target antigen to cell surface receptors expressed by antigen presenting cells.

A. A fusion protein expressed from a cDNA construct that encodes an antigen domain attached with a linker to a receptor targeting domain. The antigen domain may be attached to the amino terminus of the receptor targeting domain as shown, or may be attached to the carboxy terminus of the receptor targeting domain.

B. A fusion protein expressed from a cDNA construct that encodes the HIV env antigen or a subdomain, is attached to the amino terminus of the CD154 extracellular domain.

C. A fusion protein expressed from a cDNA construct that encodes the HIV env antigen or a subdomain, is attached to the amino terminus of a single chain Fv specific for CD40.

D. A fusion protein expressed from a cDNA construct as in C, except that the scFv that binds CD40 is oriented with the light chain variable region (V_L) attached to the carboxy-terminus of the heavy chain variable region (V_H).

E. A fusion protein expressed from a cDNA construct that encodes the HIV env antigen or a subdomain, is attached to a camelid variable region (V_{HH}) that binds CD40.

F. A fusion protein expressed from a cDNA construct that encodes the HIV env antigen or a subdomain, is attached to a peptide that binds CD40.

Figure 2.

A. Sequence of two cDNAs encoding HIV gp120-V3 loop/CD154 long form extracellular domain fusion proteins.

The sequence of a cDNA construct and corresponding fusion protein encoding the HIV V3 loop from gp120 with a (ProAspPro) linker (SEQUENCE ID NO.: 17 [DNA] OR SEQUENCE ID NO.: 25 [FUSION PROTEIN]) or a $(Gly_4Ser)_3$ linker (SEQ. ID NO.: 16 [DNA] OR SEQ. ID NO.:24 [FUSION PROTEIN]) fused to the CD154 extracellular domain encoded between amino acids 48 (Arg)-261(Leu), with an additional (Glu) residue at the carboxyl end of the protein, not found in wild type CD154. The sequence of the fusion protein is indicated using the three-letter amino acid code convention, above each codon of the open reading frame. Relevant restriction sites are indicated on the drawing and the nucleotides encoding sites at domain fusion junctions are displayed in boldface type, while the first codon of each fused domain is indicated in underlined, italicized type. The protein domains are labeled above the relevant position in the sequence. The nucleotide number is indicated in the left margin with a designation for the PDP linker form or the G4S linker form.

B. Sequence of two cDNAs encoding HIV V3 loop-CD154 short form extracellular domain fusion proteins.

The two HIV V3 loop constructs with alternate linkers, either (ProAspPro) (SEQUENCE ID NO.:19 [DNA] OR SEQUENCE ID NO.: 27 [FUSION PROTEIN]) or $(Gly_4Ser)_3$ (SEQUENCE ID NO.: 18 [DNA] OR SEQUENCE ID NO.: 26 [FUSION PROTEIN])

were also fused to the short form of the CD154 extracellular domain encoded from amino acids 108 (Glu)-261 (Leu), plus an extra glutamic acid residue at the carboxy terminus, not encoded by wild type CD154. All sequences are labeled as described for Figure 2A.

Figure 3.

A. Sequence of two HIV gp120env-CD154 long form extracellular domain cDNA and the predicted fusion proteins.

The sequence of a cDNA construct and corresponding fusion protein encoding the HIV gp120 with a (ProAspPro) linker (SEQ. ID NO.: 13 [DNA] OR SEQ. ID NO.: 21 [FUSION PROTEIN]) or a $(Gly_4Ser)_3$ linker (SEQ. ID NO.: 12 [DNA] OR SEQ. ID NO.: 20 [FUSION PROTEIN]) fused to the CD154 extracellular domain (Long Form) encoded between amino acids 48 (Arg)-261(Leu) + (Glu). All sequences are labeled as described for Figure 2A.

B. Sequence of two HIV gp120env-CD154 short form extracellular domain cDNAs and the predicted fusion proteins.

The sequence of a cDNA construct and corresponding fusion protein encoding the HIV gp120 with a (ProAspPro) linker (SEQ. ID NO.: 15 [DNA] or SEQ. ID NO.: 23 [fusion protein]) or a (Gly4Ser)3 linker (SEQ. ID NO.: 14 [DNA] or SEQ. ID NO.: 22 [fusion protein]) fused to the short form of the CD154 extracellular domain encoded between amino acids 108 (Glu)-261 (Leu) + (Glu). All sequences are labeled as described for Figure 2A.

BEST MODES FOR CARRYING OUT THE INVENTION:

This invention relates to improved vaccines comprising one or more antigens attached to a domain that targets at least one cell surface receptor. The vaccine may be delivered either as a protein, as a DNA plasmid, or by a viral vector. The expression of the DNA after injection of the plasmid or viral vector *in vivo* results in the secretion of the antigen(s) attached to a targeting domain, directing the antigen(s) to a cell surface receptor. Receptor-mediated internalization of the antigen into the endocytic compartment of cells that express the receptor enhances the presentation of antigenic peptides by MHC class II molecules that circulate through this compartment.

Presentation of antigenic peptides by MHC class I molecules is mediated by the cells expressing the DNA vaccine, and is enhanced in cells that internalize the antigen-targeting domain fusion protein by movement of the fusion protein from the endocytic compartment into the cytoplasm. The activation of antigen-specific CD4+ T cells and CD8+ T cells is increased, resulting in better humoral and cellular immune responses.

The preferred receptor(s) chosen for antigen targeting are those expressed by antigen presenting cells (APC), such as dendritic cells. Desirable receptors for targeting include but are not limited to CD80, CD86, CD83, CD40, CD32, CD64, Dec 205, Flt3, and ICOS ligand. The CD40 receptor is a preferred receptor for antigen targeting, since signals from CD40 regulate activation and differentiation of APC. Fusion proteins of antigen and CD154 (CD40 ligand) combine the functions of antigen targeting and activation of APC by simultaneous delivery of CD40 signals.

The preferred antigen(s) for receptor targeting are HIV-1 and HIV-2 viral antigens, since vaccines have not been effective in protecting against virulent viral isolates. Attachment of HIV-1 gp160 or gp120 extracellular domain to CD154 extracellular domain stabilizes the trimeric structure of HIV-1 env. However, the invention is not limited to HIV env antigens, since improved immune responses to vaccines are needed to provide long-lasting protection against infection with high doses of pathogenic microorganisms or against tumors.

Thus the structure of the invention's main embodiment is a DNA plasmid encoding the extracellular domain of HIV-1 env gp160 attached to the CD154 extracellular domain.

The fusion protein expressed from this DNA plasmid a) stabilizes the trimeric structure of HIV-1 env, b) directs the HIV-1 antigen into the MHC class II compartment of CD40 positive cells, and c) selectively activates the CD40 receptor to increase APC functional activity.

The main embodiment of the invention encodes a stable trimer that expresses the major cross-neutralization epitopes of HIV-1 env while masking the internal env

epitopes that are not involved in virus neutralization. Antigenic peptides of HIV env are presented by MHC class I molecules by cells that express the DNA, while antigenic peptides of HIV env are presented by MHC class II molecules in CD40 positive cells that internalize the trimeric antigen-CD154 fusion protein. Activation of the CD40 receptor on cells bound by the antigen-CD154 fusion protein increases the specific immune response due to increased production of IL12 and increased expression of costimulatory molecules CD80 and CD86.

An improved DNA vaccine for AIDS comprising the extracellular domain of HIV-1 gp160, HIV-1 gp120, or a subdomain of these antigens fused to the extracellular domain of CD154 is described. Alternative embodiments of the invention use a smaller portion of the CD154 molecule composed of an 18 kDa subunit from Glu-108 to Leu-261 (Mazzei G.J. et al, J. Biol. Chem. 270: 7025-7028, 1995). The extracellular domain of gp160 can also be shortened by removing the gp41 domain, removing the V1 and V2 domains, or mutating the glycosylation sites without damaging the conformational structure of the HIV-1 envelope (Kwong P.D. et al, Nature 393: 648-659, 1998). These changes could further improve the activity of the vaccine, since the V1 and V2 loops, and the carbohydrate structures are thought to be exposed, clade specific epitopes that prevent or dilute the immune response to important cross-neutralization epitopes for diverse clades of HIV-1. Linkers between gp160 and CD154 can also be used. Thus, alternative embodiments of the invention minimize the CD154 domain, remove gp41, V1, V2, or glycosylation sites of gp160. This invention also envisions DNA vaccines comprising other HIV-1 antigens and antigens from alternative isolates of HIV-1, fused to the extracellular domain of CD154.

Delivery of antigen(s) to the CD40 receptor may use anti-CD40 scFv instead of CD154. Single antibody variable regions (V_{HH}) or peptides that bind CD40 are also included in the scope of the invention.

Antigen targeting to receptors is not limited to the CD40 receptor. Alternative receptors preferred for targeting include CD80, CD86, Dec205, ICOS ligand, Flt3, Fc receptors, and CD83. All cell surface receptors are envisioned by this invention. Receptors may be targeted by ligands, scFv molecules, single variable regions or

peptides. Additional methods of attachment of antigen(s) to receptor targeting domains are envisioned, including chemical linkages of subunits, disulfide bonds, or noncovalent attachments such as leucine zipper motifs and the like. The invention contemplates injection of protein, injection of DNA plasmids, or viral vectors encoding the molecules comprising one or more antigens linked to a receptor-binding domain.

Antigens targeted to cell surface receptors are not limited to HIV gp160 antigens. Other antigens, including tumor antigens, parasite antigens, bacterial antigens, and viral antigens are included in the scope of the invention.

The invention also envisions delivery of antigens to cell surface receptors in order to induce antigen-specific tolerance or nonresponsiveness. For this application, an autoantigen would be chosen and the vaccine would be used to treat autoimmune disease.

The invention also envisions antigen(s) that are natural components of the body, such as tumor-associated antigens, where an immune response to the antigen(s) breaks tolerance to the antigen, resulting in a change in immune homeostasis.

The following examples describe particular embodiments of the invention but are not meant to limit its scope.

EXAMPLE 1

A preferred embodiment of the DNA vaccine includes an amino-terminal secretory signal peptide sequence upstream and adjacent to a cDNA sequence cassette encoding the desired antigen. This molecule is then fused to the extracellular domain of CD154 or to a portion of the extracellular domain of CD154 which retains the ability to bind CD40, or to an scFv targeted to CD40, to create a fusion protein expression cassette that targets the antigen to the antigen presenting cell through the CD40 receptor as diagrammed in Figure 1. The expression cassette is inserted into an appropriate mammalian expression vector or virus to achieve high level expression of the fusion protein either *in vitro* or *in vivo*.

The leader peptide is encoded on complementary oligonucleotides with a single-stranded HindIII cohesive end at the 5' terminus, and a BglII cohesive end at the 3'

terminus. The sense oligonucleotide is designated SEQUENCE ID NO: 1 or HBLPS and the sequence is as follows:

5'agcttgccgccatgctgtatacctctcagctgttaggactacttctgttttggatctcggcttcga-3'.

The antisense oligonucleotide is designated SEQUENCE ID NO: 2 or HBLPAS and the sequence is as follows:

5'gatctcgaagcccgagatccaaaacagaagtagtcctaacagctgagaggtatacagcatggcggca-3'. The two molecules anneal to one another except at the overhanging nucleotides indicated in boldface type. Alternative embodiments could include other secretory signal peptides or localization sequences.

The extracellular domain of human CD154 was PCR amplified using cDNA generated with random primers and RNA from human T lymphocytes activated with PHA (phytohemagglutinin). Two different fusion junctions were designed which resulted in a short or truncated form (form S4) including amino acids 108 (Glu)-261 (Leu)+(Glu), and a long or complete form (form L2) including amino acids 48 (Arg) - 261 (Leu)+(Glu) of the extracellular domain of CD154. The sense primer which fuses the extracellular domain to the targeted antigen includes a BamHI site for cloning that introduces the peptide sequence PDP or (ProAspPro) at the fusion junction and can also encode a linker peptide such as $(Gly_4Ser)_3$ to separate the antigen from the extracellular domain. The oligonucleotide primers used in amplifying the short form (S4) of the CD154 extracellular domain encoding amino acids 108 (Glu)-261 (Leu)+(Glu) are as follows:

The sense primer is designated SEQUENCE ID NO: 3 or CD154BAM108 and encodes a 34 mer with the following sequence : 5'-gtt gtc gga tcc aga aaa cag ctt tga aat gca a-3' , while the antisense primer is designated SEQUENCE ID NO: 4 or CD154XBA and encodes a 44 mer with the following sequence: 5'-gtt gtt tct aga tta tca ctc gag ttt gag taa gcc aaa gga cg-3'.

The oligonucleotide primers used in amplifying the long form (L2) of the CD154 extracellular domain encoding amino acids 48 (Arg)-261 (Leu) + (Glu), are as follows: The sense primer is identified as SEQUENCE ID NO: 5 or CD154 BAM48 and encodes a 35 mer with the following sequence: 5'-gtt gtc gga tcc aag aag gtt gga caa gat aga ag-3', while the antisense primer is also SEQUENCE ID NO: 4 or CD154XBA encoding the 44 mer: 5'-gtt gtt tct aga tta tca ctc gag ttt gag taa gcc aaa gga cg-3'.

A variety of different antigens can be encoded on cDNA cassettes to be inserted between the leader peptide cassette and the CD40 targeted domain (such as a truncated or complete CD154 extracellular domain or a CD40 specific scFv). In a preferred embodiment of the invention, the cDNA antigen encoded by the vaccine is the HIV-1 gp 120 or a fragment of this antigen, such as the V3 loop. The primer sets used to amplify the complete gp120 domain include the sense primer SEQUENCE ID NO: 6 or GP120Bgl2f 5'-gga tat tga tga gat cta gtg cta cag-3' and one of two antisense primers encoding different linkers. Either the antisense primer encoding the ProAspPro linker, identified as SEQUENCE ID NO: 7 or GP120PDPr 5'-gaa cac agc tcc tat tgg atc cgg tct ttt ttc tct ttg cac-3' or the antisense primer encoding the $(Gly_4Ser)_3$ linker, identified as SEQUENCE ID NO: 8 or GP120G4Sr 5'-cct gca tgg atc cga tcc gcc acc tcc aga acc tcc acc tcc tga acc gcc tcc ccc tct ttt ttc tct ttg cac tgt tct tct ctt tgc-3' were used to amplify the gp120 domain with the desired linker attached. PV75Kgp160(89.6) DNA was used as template in PCR reactions. Alternatively, other isolates or sequence variants of gp120 or gp160 are available and can be substituted to create novel fusion cassettes. PCR amplification reactions were performed using cloned plasmid DNA as template (approximately 45 ng), 3 mM $MgCl_2$, 0,3 MM dNTPs, 1/10 volume 10X reaction buffer supplied by the manufacturer, 10 pmol sense primer, 10 pmol antisense primer, and 2.5 units TAQ polymerase (Takara Pharmaceuticals) in a total reaction volume of 50 µl. The amplification profile included an initial 4 minute 94°C denaturation, followed by a 30 cycle program of 50°C annealing for 30 seconds, 72°C extension for 30 seconds, and 94°C denaturation for 30 seconds. PCR fragments were purified by ethanol precipitation, resuspended in 30 µl ddH_2O and 10 µl was digested with BglII (Roche) restriction endonuclease in a 20 µl reaction volume at 37°C for 3 hours. Fragments were gel purified, purified using QIAEX kits according to the manufacturer's instructions (QIAGEN, San Diego, CA), and ligated along with the annealed leader peptide oligonucleotides to HindIII-BamHI digested expression vector already containing the CD154 extracellular domain as a BamHI-XbaI fragment. Recombinant clones were screened for the correct orientation and presence of inserts, and the resulting positive clones were verified by DNA sequencing using an ABI 310 sequence analyzer and the ABI Prism Dye Terminator Reaction Chemistry. The final fusion cassette encodes the synthetic leader peptide fused to the HIV gp120 domain with either a (ProAspPro) linker

or a $(Gly_4Ser)_3$ linker, and then to the CD154 extracellular domain long (Figure 3A) or short (Figure 3B) form to create the embodiments of example 1.

EXAMPLE 2

In an alternative preferred embodiment, the V1 and V2 domains of gp120 are removed and only the V3 loop domain from HIV gp 120 is encoded on a BglII-BamHI fragment and fused to the signal peptide and the CD154 extracellular domain to create the vaccine, as illustrated in Figure 2A and B. This antigen domain is separated from the CD154 short (Figure 2B) or long extracellular domain (Figure 2A) by a peptide linker encoding the amino acids (ProAspPro), or a longer peptide linker encoding the amino acids $(Gly_4Ser)_3$.

The V3 loop was PCR amplified from pV75 (gp 89.6), a plasmid containing HIV gp120 from isolate LAV, using the following primer set:

The antisense primer encoding a ProAspPro linker is SEQUENCE ID NO: 9 or V3PDPr

5'-gtt att cca tgg atc cgg act aat ctt aca atg tgc ttg-3'

The sense primer fusing the antigen to the signal peptide is SEQUENCE ID NO: 10 or V3Bgl2f

5'-gta cag cta aat aga tct gta gta att aat tg-3'

The antisense primer encoding a $(Gly_4Ser)_3$ linker is SEQUENCE ID NO: 11 or V3G4Sr

5'-ggt gca tgg atc cga acc tcc acc gcc aga tcc acc gcc tcc tga ggc acc gcc acc act aat gtt aca atg tgc ttg ttg tct tat atc tcc-3'.

Amplification, digestion, purification, and ligation conditions were identical to those described above for the full-length gp120 domain. The final fusion cassettes encode the HIV gp120-V3 loop with either a (ProAspPro) linker or a $(Gly_4Ser)_3$ linker fused to either the CD154 extracellular domain as diagrammed in Figure 2A for the long form, and Figure 2B for the short form of the CD40 binding domain.

Other antigens and linkers can be substituted to create alternative vaccines by construction of the appropriate cDNA cassettes encoding the desired domains and attaching them to the CD154 extracellular domain. Because of the high degree of sequence variation among HIV isolates, alternative sequences might be incorporated as needed to target particular clades. Other viral antigens such as HIV tat or their

subdomains can be substituted for the HIV domains described here. Similarly, an alternate APC targeted domain can be substituted for the CD40 binding domain, such as a domain which binds to CD80 or CD86, or to ICOS ligand, or to one of several other cell surface receptors expressed on antigen presenting cells. Surface receptors that internalize readily are preferred over receptors that contain multiple transmembrane domains and do not internalize readily such as G-protein coupled chemokine receptors.

CLAIMS: We claim:

1. A vaccine comprising one or more antigens linked to a domain that binds at least one receptor.
2. A vaccine of claim 1 where said receptor is CD40.
3. A vaccine of claim 1 where said domain is CD154 or a portion of CD154.
4. A vaccine of claim 1 where said domain is a single chain Fv that binds CD40.
5. A vaccine of claim 1 where said domain binds to one or more receptors selected from the group consisting of CD80, CD86, CD32, CD64, CD83, ICOS ligand, Flt3, CD10, CD11, CD14, CD15, CD16, CD18, CD19, CD20, CD21, CD22, CD23, CD37, CD38, CD39, CD43, CD56, CD58, CD72, CD75, CD76, CD77, CD78, and cytokine/growth factor receptors.
6. A vaccine of claim 1 where said antigen is HIV-1 gp160 or a portion of HIV-1 gp160.
7. A vaccine of claim 1 where said antigen is a tumor antigen or a microbial antigen.
8. A DNA expression plasmid encoding a vaccine comprising one or more antigens linked to a domain that binds at least one receptor.
9. A DNA expression plasmid of claim 8 encoding a vaccine where said receptor is CD40.
10. A DNA expression plasmid of claim 8 encoding a vaccine where said domain is CD154 or a portion of CD154.
11. A DNA expression plasmid of claim 8 encoding a vaccine where said domain is a single chain Fv that binds CD40.
12. A DNA expression plasmid of claim 8 encoding a vaccine where said domain binds to one or more antigens selected from the group consisting of CD80, CD86, CD32, CD64, CD83, ICOS ligand, Flt3, CD10, CD11, CD14, CD15, CD16, CD18, CD19, CD20, CD21, CD22, CD23, CD37, CD38, CD39, CD43, CD56, CD58, CD72, CD75, CD76, CD77, CD78, and cytokine/growth factor receptors.
13. A DNA expression plasmid of claim 8 encoding a vaccine where said antigen is HIV-1 gp160 or a portion of HIV-1 gp160.
14. A DNA expression plasmid of claim 8 encoding a vaccine where said antigen is a tumor antigen or a microbial antigen.

Figure 1.
Fusion Proteins that Target Antigen to APC

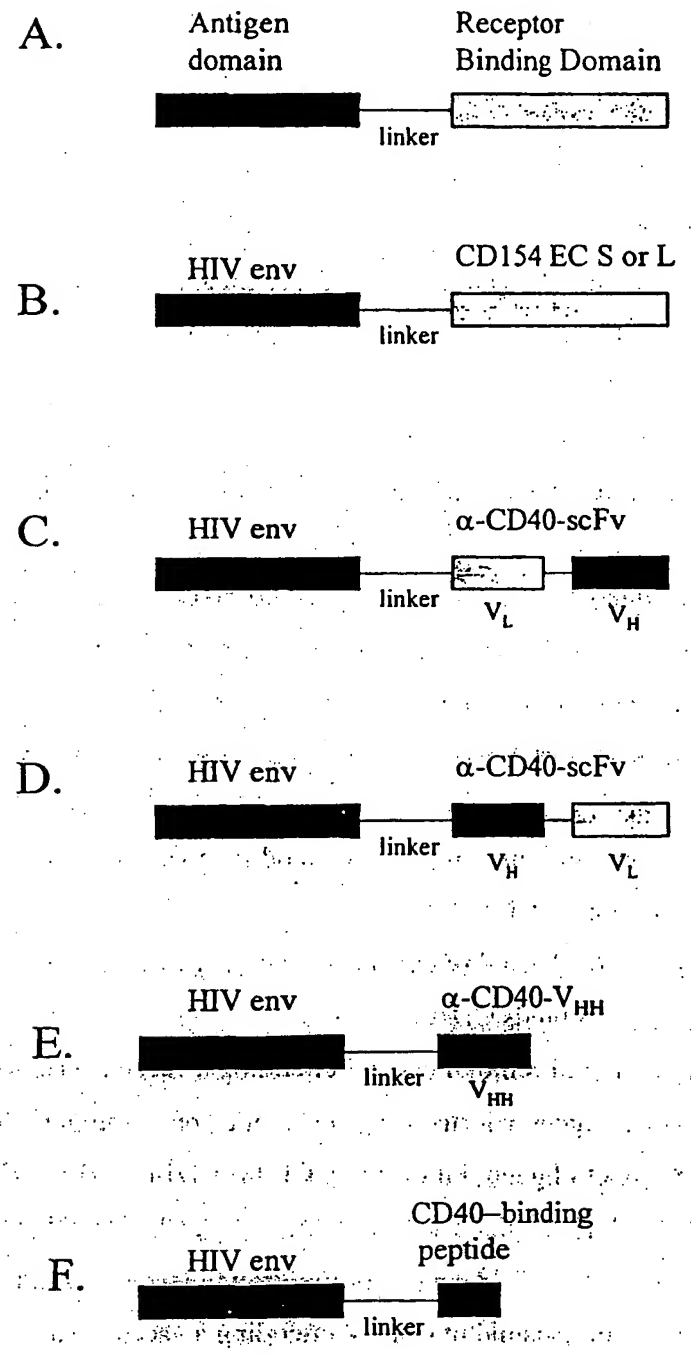

Figure 2A.

Sequence and translation of two cDNAs encoding HIV gp120 V3 loop-CD154 LONG form extracellular domain fusion proteins.

```
        HindIII
        ~~~~~~~~        Signal Peptide
                        Met Leu Tyr Thr Ser Gln Leu Leu Gly Leu Leu
  1     AAG CTT GCC GCC ATG CTG TAT ACC TCT CAG CTG TTA GGA CTA CTT
                                  BglII
                                  ~~~~~~~~ HIVgp120-V3 loop
        Leu Phe Trp Ile Ser Ala Ser Arg Ser Val Val Ile Asn Cys Thr
 46     CTG TTT TGG ATC TCG GCT TCG AGA TCT GTA GTA ATT AAT TGT ACA
        Arg Pro Asn Asn Asn Thr Arg Arg Arg Leu Ser Ile Gly Pro Gly
 91     AGA CCC AAC AAC AAT ACA AGA AGA AGG TTA TCT ATA GGA CCA GGG
        Arg Ala Phe Tyr Ala Arg Arg Asn Ile Ile Gly Asp Ile Arg Gln
136     AGA GCA TTT TAT GCA AGA AGA AAC ATA ATA GGA GAT ATA AGA CAA
        Ala His Cys Asn Ile Ser
181     GCA CAT TGT AAC ATT AGT
        ProAspPro Linker
           BamHI
           ~~~~~~~~
        +-------------+
        | Pro Asp Pro |
199     | CCG GAT CCA |
        +-------------+

        OR (Gly4Ser)3 Linker                                    BamHI
                                                                ~~~~~~~~
        +-------------------------------------------------------------+
        | Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Pro |
199     | GGT GGC GGT GGC TCA GGA GGC GGT GGA TCT GGC GGT GGA GGT TCG GAT CCA |
        +-------------------------------------------------------------+

        CD154 LONG extracellular domain
208PDP  Arg Arg Leu Asp Lys Ile Glu
250GS   AGA AGG TTG GAC AAG ATA GAA
229PDP  Asp Glu Arg Asn Leu His Glu Asp Phe Val Phe Met Lys Thr Ile
271GS   GAT GAA AGG AAT CTT CAT GAA GAT TTT GTA TTC ATG AAA ACG ATA
274PDP  Gln Arg Cys Asn Thr Gly Glu Arg Ser Leu Ser Leu Leu Asn Cys
316GS   CAG AGA TGC AAC ACA GGA GAA AGA TCC TTA TCC TTA CTG AAC TGT
319PDP  Glu Glu Ile Lys Ser Gln Phe Glu Gly Phe Val Lys Asp Ile Met
361GS   GAG GAG ATT AAA AGC CAG TTT GAA GGC TTT GTG AAG GAT ATA ATG
364PDP  Leu Asn Lys Glu Glu Thr Lys Lys Glu Asn Ser Phe Glu Met Gln
406GS   TTA AAC AAA GAG GAG ACG AAG AAA GAA AAC AGC TTT GAA ATG CAA
409PDP  Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala His Val Ile Ser Glu
451GS   AAA GGT GAT CAG AAT CCT CAA ATT GCG GCA CAT GTC ATA AGT GAG
454PDP  Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu Lys Gly
496GS   GCC AGC AGT AAA ACA ACA TCT GTG TTA CAG TGG GCT GAA AAA GGA
499PDP  Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly Lys
541GS   TAC TAC ACC ATG AGC AAC AAC TTG GTA ACC CTG GAA AAT GGG AAA
544PDP  Gln Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln
586GS   CAG CTG ACC GTT AAA AGA CAA GGA CTC TAT TAT ATC TAT GCC CAA
589PDP  Val Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe
631GS   GTC ACC TTC TGT TCC AAT CGG GAA GCT TCG AGT CAA GCT CCA TTT
634PDP  Ile Ala Ser Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile
676GS   ATA GCC AGC CTC TGC CTA AAG TCC CCC GGT AGA TTC GAG AGA ATC
679PDP  Leu Leu Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly
721GS   TTA CTC AGA GCT GCA AAT ACC CAC AGT TCC GCC AAA CCT TGC GGG
724PDP  Gln Gln Ser Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro Gly
766GS   CAA CAA TCC ATT CAC TTG GGA GGA GTA TTT GAA TTG CAA CCA GGT
769PDP  Ala Ser Val Phe Val Asn Val Thr Asp Pro Ser Gln Val Ser His
811GS   GCT TCG GTG TTT GTC AAT GTG ACT GAT CCA AGC CAA GTG AGC CAT
814PDP  Gly Thr Gly Phe Thr Ser Phe Gly Leu Leu Lys Leu Glu *** ***
856GS   GGC ACT GGC TTC ACG TCC TTT GGC TTA CTC AAA CTC GAG TGA TAA
        XbaI
859PDP  ~~~~~~~
901GS   TCT AGA
```

Figure 2B.

Sequence and translation of two cDNAs encoding HIV gp120 V3 loop-CD154 SHORT form extracellular domain fusion proteins.

```
        HindIII
        ~~~~~~~~        Signal Peptide
                        Met Leu Tyr Thr Ser Gln Leu Leu Gly Leu Leu
   1    AAG CTT GCC GCC ATG CTG TAT ACC TCT CAG CTG TTA GGA CTA CTT
                                    BglII HIVgp120-V3 loop
                                    ~~~~~~~~
        Leu Phe Trp Ile Ser Ala Ser Arg Ser Val Val Ile Asn Cys Thr
  46    CTG TTT TGG ATC TCG GCT TCG AGA TCT GTA GTA ATT AAT TGT ACA
        Arg Pro Asn Asn Asn Thr Arg Arg Arg Leu Ser Ile Gly Pro Gly
  91    AGA CCC AAC AAC AAT ACA AGA AGA AGG TTA TCT ATA GGA CCA GGG
        Arg Ala Phe Tyr Ala Arg Arg Asn Ile Ile Gly Asp Ile Arg Gln
 136    AGA GCA TTT TAT GCA AGA AGA AAC ATA ATA GGA GAT ATA AGA CAA
        Ala His Cys Asn Ile Ser
 181    GCA CAT TGT AAC ATT AGT

        ProAspPro Linker
            BamHI
            ~~~~~~~
        Pro Asp Pro
 199    CCG GAT CCA

        OR (Gly4Ser)3 Linker                                    BamHI
                                                                ~~~~~~~
   Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Pro
199GGT GGC GGT GGC TCA GGA GGC GGT GGA TCT GGC GGT GGA GGT TCG GAT CCA

        CD154 SHORT extracellular domain
208PDP  Glu Asn Ser Phe Glu Met Gln
250GS   GAA AAC AGC TTT GAA ATG CAA
229PDP  Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala His Val Ile Ser Glu
271GS   AAA GGT GAT CAG AAT CCT CAA ATT GCG GCA CAT GTC ATA AGT GAG
274PDP  Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu Lys Gly
316GS   GCC AGC AGT AAA ACA ACA TCT GTG TTA CAG TGG GCT GAA AAA GGA
319PDP  Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly Lys
361GS   TAC TAC ACC ATG AGC AAC AAC TTG GTA ACC CTG GAA AAT GGG AAA
364PDP  Gln Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln
406GS   CAG CTG ACC GTT AAA AGA CAA GGA CTC TAT TAT ATC TAT GCC CAA
409PDP  Val Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe
451GS   GTC ACC TTC TGT TCC AAT CGG GAA GCT TCG AGT CAA GCT CCA TTT
454PDP  Ile Ala Ser Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile
496GS   ATA GCC AGC CTC TGC CTA AAG TCC CCC GGT AGA TTC GAG AGA ATC
499PDP  Leu Leu Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly
541GS   TTA CTC AGA GCT GCA AAT ACC CAC AGT TCC GCC AAA CCT TGC GGG
544PDP  Gln Gln Ser Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro Gly
586GS   CAA CAA TCC ATT CAC TTG GGA GGA GTA TTT GAA TTG CAA CCA GGT
589PDP  Ala Ser Val Phe Val Asn Val Thr Asp Pro Ser Gln Val Ser His
631GS   GCT TCG GTG TTT GTC AAT GTG ACT GAT CCA AGC CAA GTG AGC CAT
634GS   Gly Thr Gly Phe Thr Ser Phe Gly Leu Leu Lys Leu Glu *** ***
676GS   GGC ACT GGC TTC ACG TCC TTT GGC TTA CTC AAA CTC GAG TGA TAA
        XbaI
        ~~~~~~~
679PDP  Ser Arg
721GS   TCT AGA
```

Figure 3A.

Sequence and translation of two cDNAs encoding HIV gp120-CD154 LONG form extracellular domain fusion proteins.

```
        HindIII
        ~~~~~~~~        Signal Peptide
                        Met Leu Tyr Thr Ser Gln Leu Leu Gly Leu Leu
      1 AAG CTT GCC GCC ATG CTG TAT ACC TCT CAG CTG TTA GGA CTA CTT
                                  BglII
                                  ~~~~~~~~    HIV gp120 domain
        Leu Phe Trp Ile Ser Ala Ser Arg Ser Met Leu Leu Gly Ile Leu
     46 CTG TTT TGG ATC TCG GCT TCG AGA TCT ATG CTC CTT GGG ATA TTG
        Met Ile Cys Ser Ala Thr Glu Lys Leu Trp Val Thr Val Tyr Tyr
     91 ATG ATC TGT AGT GCT ACA GAA AAA TTG TGG GTC ACA GTC TAT TAT
        Gly Val Pro Val Trp Arg Glu Ala Thr Thr Thr Leu Phe Cys Ala
    136 GGG GTA CCT GTG TGG AGA GAA GCA ACC ACC ACT CTA TTT TGT GCA
        Ser Asp Ala Lys Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala
    181 TCA GAT GCT AAA GCC TAT GAT ACA GAG GTA CAT AAT GTT TGG GCC
        Thr His Ala Cys Val Pro Thr Asp Pro Asn Pro Gln Glu Val Val
    226 ACA CAT GCC TGT GTA CCC ACA GAC CCC AAC CCA CAA GAA GTA GTA
        Leu Gly Asn Val Thr Glu Asn Phe Asn Met Trp Lys Asn Asn Met
    271 TTG GGA AAT GTG ACA GAA AAT TTT AAC ATG TGG AAA AAT AAC ATG
        Val Asp Gln Met His Glu Asp Ile Ile Ser Leu Trp Asp Glu Ser
    316 GTA GAT CAG ATG CAT GAG GAT ATA ATC AGT TTA TGG GAT GAA AGC
        Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Thr Leu Asn
    361 CTA AAG CCA TGT GTA AAA TTA ACC CCA CTC TGT GTT ACT TTA AAT
        Cys Thr Asn Leu Asn Ile Thr Lys Asn Thr Thr Asn Pro Thr Ser
    406 TGC ACT AAT TTG AAT ATC ACT AAG AAT ACT ACT AAT CCC ACT AGT
        Ser Ser Trp Gly Met Met Glu Lys Gly Glu Ile Lys Asn Cys Ser
    451 AGC AGC TGG GGA ATG ATG GAG AAA GGA GAA ATA AAA AAT TGC TCT
        Phe Tyr Ile Thr Thr Ser Ile Arg Asn Lys Val Lys Lys Glu Tyr
    496 TTC TAT ATC ACC ACA AGC ATA AGA AAT AAG GTA AAG AAA GAA TAT
        Ala Leu Phe Asn Arg Leu Asp Val Val Pro Ile Glu Asn Thr Asn
    541 GCA CTT TTT AAT AGA CTT GAT GTA GTA CCA ATA GAA AAT ACT AAT
        Asn Thr Lys Tyr Arg Leu Ile Ser Cys Asn Thr Ser Val Ile Thr
    586 AAT ACT AAG TAT AGG TTA ATA AGT TGT AAC ACC TCA GTC ATT ACA
        Gln Ala Cys Pro Lys Val Ser Phe Gln Pro Ile Pro Ile His Tyr
    631 CAG GCC TGT CCA AAG GTA TCC TTT CAG CCA ATT CCC ATA CAT TAT
        Cys Val Pro Ala Gly Phe Ala Met Leu Lys Cys Asn Asn Lys Thr
    676 TGT GTC CCG GCT GGG TTT GCG ATG CTA AAG TGT AAC AAT AAG ACA
        Phe Asn Gly Ser Gly Pro Cys Thr Asn Val Ser Thr Val Gln Cys
    721 TTC AAT GGA TCA GGA CCA TGC ACA AAT GTC AGC ACA GTA CAA TGT
        Thr His Gly Ile Arg Pro Val Val Ser Thr Gln Leu Leu Leu Asn
    766 ACA CAT GGA ATT AGG CCA GTG GTG TCA ACT CAA CTG CTG TTA AAT
        Gly Ser Leu Ala Glu Glu Asp Ile Val Ile Arg Ser Glu Asn Phe
    811 GGC AGT CTA GCA GAA GAA GAC ATA GTA ATT AGA TCT GAA AAT TTC
        Thr Asp Asn Ala Lys Thr Ile Ile Val Gln Leu Asn Glu Ser Val
    856 ACA GAC AAT GCT AAA ACC ATA ATA GTA CAG CTA AAT GAA TCT GTA
        Val Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr Arg Arg Arg Leu
    901 GTA ATT AAT TGT ACA AGA CCC AAC AAC AAT ACA AGA AGA AGG TTA
        Ser Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg Asn Ile Ile
    946 TCT ATA GGA CCA GGG AGA GCA TTT TAT GCA AGA AGA AAC ATA ATA
        Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Ala Lys Trp
    991 GGA GAT ATA AGA CAA GCA CAT TGT AAC ATT AGT AGA GCA AAA TGG
        Asn Asn Thr Leu Gln Gln Ile Val Ile Lys Leu Arg Glu Lys Phe
   1036 AAT AAC ACT TTA CAA CAG ATA GTT ATA AAA TTA AGA GAA AAA TTT
        Arg Asn Lys Thr Ile Ala Phe Asn Gln Ser Ser Gly Gly Asp Pro
   1081 AGG AAT AAA ACA ATA GCC TTT AAT CAA TCC TCA GGA GGG GAC CCA
        Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr
   1126 GAA ATT GTA ATG CAC AGT TTT AAT TGT GGA GGG GAA TTC TTC TAC
        Cys Asn Thr Ala Gln Leu Phe Asn Ser Thr Trp Asn Val Thr Gly
   1171 TGT AAT ACA GCA CAA CTG TTT AAT AGT ACT TGG AAT GTT ACT GGA
        Gly Thr Asn Gly Thr Glu Gly Asn Asp Ile Ile Thr Leu Gln Cys
```

Figure 3A (continued).

Sequence and translation of two cDNAs encoding HIV gp120-CD154 LONG form extracellular domain fusion proteins.

```
1216  GGG ACA AAT GGC ACT GAA GGA AAT GAC ATA ATC ACA CTC CAA TGC
      Arg Ile Lys Gln Ile Ile Asn Met Trp Gln Lys Val Gly Lys Ala
1261  AGA ATA AAA CAA ATT ATA AAT ATG TGG CAG AAA GTA GGA AAA GCA
      Met Tyr Ala Pro Pro Ile Thr Gly Gln Ile Arg Cys Ser Ser Asn
1306  ATG TAT GCC CCT CCC ATC ACA GGA CAA ATT AGA TGT TCA TCA AAT
      Ile Thr Gly Leu Leu Leu Thr Arg Asp Gly Gly Asn Ser Thr Glu
1351  ATT ACA GGG CTG CTA CTA ACA AGA GAT GGA GGT AAT AGT ACT GAG
      Thr Glu Thr Glu Ile Phe Arg Pro Gly Gly Gly Asp Met Arg Asp
1396  ACT GAG ACT GAG ATC TTC AGA CCT GGA GGA GGA GAT ATG AGG GAC
      Asn Trp Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val Arg Ile Glu
1441  AAT TGG AGA AGT GAA TTA TAT AAA TAT AAA GTA GTA AGA ATT GAA
      Pro Ile Gly Val Ala Pro Thr Arg Ala Lys Arg Arg Thr Val Gln
1486  CCA ATA GGA GTA GCA CCC ACC AGG GCA AAG AGA AGA ACA GTG CAA
      Arg Glu Lys Arg
1531  AGA GAA AAA AGA
```

(Gly$_4$Ser)$_3$ linker BamHI

```
                                                        ~~~~~~~~
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Pro
1543  GGG GGA GGC GGT TCA GGA GGT GGA GGT TCT GGA GGT GGC GGA TCG GAT CCA
```

OR ProAspPro linker

BamHI

```
1543  Pro Asp Pro
      CCG GAT CCA
```

CD154 LONG FORM Extracellular Domain

```
1594GS      Arg Arg Leu Asp Lys Ile Glu Asp Glu
1552PDP     AGA AGG TTG GAC AAG ATA GAA GAT GAA
1621GS      Arg Asn Leu His Glu Asp Phe Val Phe Met Lys Thr Ile Gln Arg
1579PDP     AGG AAT CTT CAT GAA GAT TTT GTA TTC ATG AAA ACG ATA CAG AGA
1666GS      Cys Asn Thr Gly Glu Arg Ser Leu Ser Leu Leu Asn Cys Glu Glu
1624PDP     TGC AAC ACA GGA GAA AGA TCC TTA TCC TTA CTG AAC TGT GAG GAG
1711GS      Ile Lys Ser Gln Phe Glu Gly Phe Val Lys Asp Ile Met Leu Asn
1669PDP     ATT AAA AGC CAG TTT GAA GGC TTT GTG AAG GAT ATA ATG TTA AAC
1756GS      Lys Glu Glu Thr Lys Lys Glu Asn Ser Phe Glu Met Gln Lys Gly
1714PDP     AAA GAG GAG ACG AAG AAA GAA AAC AGC TTT GAA ATG CAA AAA GGT
1801GS      Asp Gln Asn Pro Gln Ile Ala Ala His Val Ile Ser Glu Ala Ser
1759PDP     GAT CAG AAT CCT CAA ATT GCG GCA CAT GTC ATA AGT GAG GCC AGC
1846GS      Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu Lys Gly Tyr Tyr
1804PDP     AGT AAA ACA ACA TCT GTG TTA CAG TGG GCT GAA AAA GGA TAC TAC
1891GS      Thr Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly Lys Gln Leu
1849PDP     ACC ATG AGC AAC AAC TTG GTA ACC CTG GAA AAT GGG AAA CAG CTG
1936GS      Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln Val Thr
1894PDP     ACC GTT AAA AGA CAA GGA CTC TAT TAT ATC TAT GCC CAA GTC ACC
1981GS      Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe Ile Ala
1939PDP     TTC TGT TCC AAT CGG GAA GCT TCG AGT CAA GCT CCA TTT ATA GCC
2026GS      Ser Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile Leu Leu
1984PDP     AGC CTC TGC CTA AAG TCC CCC GGT AGA TTC GAG AGA ATC TTA CTC
2071GS      Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln Gln
2029PDP     AGA GCT GCA AAT ACC CAC AGT TCC GCC AAA CCT TGC GGG CAA CAA
2116GS      Ser Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser
2074PDP     TCC ATT CAC TTG GGA GGA GTA TTT GAA TTG CAA CCA GGT GCT TCG
2161GS      Val Phe Val Asn Val Thr Asp Pro Ser Gln Val Ser His Gly Thr
2119PDP     GTG TTT GTC AAT GTG ACT GAT CCA AGC CAA GTG AGC CAT GGC ACT
                                                        XbaI
2206GS      Gly Phe Thr Ser Phe Gly Leu Leu Lys Leu Glu *** *** Ser Arg
2164PDP     GGC TTC ACG TCC TTT GGC TTA CTC AAA CTC GAG TGA TAA TCT AGA
```

Figure 3B.

Sequence and translation of two cDNAs encoding HIV gp120-CD154 short form extracellular domain fusion proteins.

HindIII
~~~~~~~~ *Signal Peptide*

```
                    Met Leu Tyr Thr Ser Gln Leu Leu Gly Leu Leu
  1 AAG CTT GCC GCC ATG CTG TAT ACC TCT CAG CTG TTA GGA CTA CTT
                                  BglII
                                  ~~~~~~~~   HIV gp120 domain
    Leu Phe Trp Ile Ser Ala Ser Arg Ser Met Leu Leu Gly Ile Leu
 46 CTG TTT TGG ATC TCG GCT TCG AGA TCT ATG CTC CTT GGG ATA TTG
    Met Ile Cys Ser Ala Thr Glu Lys Leu Trp Val Thr Val Tyr Tyr
 91 ATG ATC TGT AGT GCT ACA GAA AAA TTG TGG GTC ACA GTC TAT TAT
    Gly Val Pro Val Trp Arg Glu Ala Thr Thr Thr Leu Phe Cys Ala
136 GGG GTA CCT GTG TGG AGA GAA GCA ACC ACC ACT CTA TTT TGT GCA
    Ser Asp Ala Lys Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala
181 TCA GAT GCT AAA GCC TAT GAT ACA GAG GTA CAT AAT GTT TGG GCC
    Thr His Ala Cys Val Pro Thr Asp Pro Asn Pro Gln Glu Val Val
226 ACA CAT GCC TGT GTA CCC ACA GAC CCC AAC CCA CAA GAA GTA GTA
    Leu Gly Asn Val Thr Glu Asn Phe Asn Met Trp Lys Asn Asn Met
271 TTG GGA AAT GTG ACA GAA AAT TTT AAC ATG TGG AAA AAT AAC ATG
    Val Asp Gln Met His Glu Asp Ile Ile Ser Leu Trp Asp Glu Ser
316 GTA GAT CAG ATG CAT GAG GAT ATA ATC AGT TTA TGG GAT GAA AGC
    Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Thr Leu Asn
361 CTA AAG CCA TGT GTA AAA TTA ACC CCA CTC TGT GTT ACT TTA AAT
    Cys Thr Asn Leu Asn Ile Thr Lys Asn Thr Thr Asn Pro Thr Ser
406 TGC ACT AAT TTG AAT ATC ACT AAG AAT ACT ACT AAT CCC ACT AGT
    Ser Ser Trp Gly Met Met Glu Lys Gly Glu Ile Lys Asn Cys Ser
451 AGC AGC TGG GGA ATG ATG GAG AAA GGA GAA ATA AAA AAT TGC TCT
    Phe Tyr Ile Thr Thr Ser Ile Arg Asn Lys Val Lys Lys Glu Tyr
496 TTC TAT ATC ACC ACA AGC ATA AGA AAT AAG GTA AAG AAA GAA TAT
    Ala Leu Phe Asn Arg Leu Asp Val Val Pro Ile Glu Asn Thr Asn
541 GCA CTT TTT AAT AGA CTT GAT GTA GTA CCA ATA GAA AAT ACT AAT
    Asn Thr Lys Tyr Arg Leu Ile Ser Cys Asn Thr Ser Val Ile Thr
586 AAT ACT AAG TAT AGG TTA ATA AGT TGT AAC ACC TCA GTC ATT ACA
    Gln Ala Cys Pro Lys Val Ser Phe Gln Pro Ile Pro Ile His Tyr
631 CAG GCC TGT CCA AAG GTA TCC TTT CAG CCA ATT CCC ATA CAT TAT
    Cys Val Pro Ala Gly Phe Ala Met Leu Lys Cys Asn Asn Lys Thr
676 TGT GTC CCG GCT GGG TTT GCG ATG CTA AAG TGT AAC AAT AAG ACA
    Phe Asn Gly Ser Gly Pro Cys Thr Asn Val Ser Thr Val Gln Cys
721 TTC AAT GGA TCA GGA CCA TGC ACA AAT GTC AGC ACA GTA CAA TGT
    Thr His Gly Ile Arg Pro Val Val Ser Thr Gln Leu Leu Leu Asn
766 ACA CAT GGA ATT AGG CCA GTG GTG TCA ACT CAA CTG CTG TTA AAT
    Gly Ser Leu Ala Glu Glu Asp Ile Val Ile Arg Ser Glu Asn Phe
811 GGC AGT CTA GCA GAA GAA GAC ATA GTA ATT AGA TCT GAA AAT TTC
    Thr Asp Asn Ala Lys Thr Ile Ile Val Gln Leu Asn Glu Ser Val
856 ACA GAC AAT GCT AAA ACC ATA ATA GTA CAG CTA AAT GAA TCT GTA
    Val Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr Arg Arg Arg Leu
901 GTA ATT AAT TGT ACA AGA CCC AAC AAC AAT ACA AGA AGA AGG TTA
    Ser Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg Asn Ile Ile
946 TCT ATA GGA CCA GGG AGA GCA TTT TAT GCA AGA AGA AAC ATA ATA
    Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Ala Lys Trp
991 GGA GAT ATA AGA CAA GCA CAT TGT AAC ATT AGT AGA GCA AAA TGG
    Asn Asn Thr Leu Gln Gln Ile Val Ile Lys Leu Arg Glu Lys Phe
1036 AAT AAC ACT TTA CAA CAG ATA GTT ATA AAA TTA AGA GAA AAA TTT
    Arg Asn Lys Thr Ile Ala Phe Asn Gln Ser Ser Gly Gly Asp Pro
1081 AGG AAT AAA ACA ATA GCC TTT AAT CAA TCC TCA GGA GGG GAC CCA
    Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr
1126 GAA ATT GTA ATG CAC AGT TTT AAT TGT GGA GGG GAA TTC TTC TAC
    Cys Asn Thr Ala Gln Leu Phe Asn Ser Thr Trp Asn Val Thr Gly
1171 TGT AAT ACA GCA CAA CTG TTT AAT AGT ACT TGG AAT GTT ACT GGA
```
```

Figure 3B (Continued).
Sequence and translation of two cDNAs encoding HIV gp120-CD154 short form extracellular domain fusion proteins.

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        Arg Ile Lys Gln Ile Ile Asn Met Trp Gln Lys Val Gly Lys Ala
1261   AGA ATA AAA CAA ATT ATA AAT ATG TGG CAG AAA GTA GGA AAA GCA
        Met Tyr Ala Pro Pro Ile Thr Gly Gln Ile Arg Cys Ser Ser Asn
1306   ATG TAT GCC CCT CCC ATC ACA GGA CAA ATT AGA TGT TCA TCA AAT
        Ile Thr Gly Leu Leu Leu Thr Arg Asp Gly Gly Asn Ser Thr Glu
1351   ATT ACA GGG CTG CTA CTA ACA AGA GAT GGA GGT AAT AGT ACT GAG
                    BglII
                    ~~~~~~~~
        Thr Glu Thr Glu Ile Phe Arg Pro Gly Gly Gly Asp Met Arg Asp
1396   ACT GAG ACT GAG ATC TTC AGA CCT GGA GGA GGA GAT ATG AGG GAC
        Asn Trp Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val Arg Ile Glu
1441   AAT TGG AGA AGT GAA TTA TAT AAA TAT AAA GTA GTA AGA ATT GAA
        Pro Ile Gly Val Ala Pro Thr Arg Ala Lys Arg Arg Thr Val Gln
1486   CCA ATA GGA GTA GCA CCC ACC AGG GCA AAG AGA AGA ACA GTG CAA
        Arg Glu Lys Arg
1531   AGA GAA AAA AGA

        (Gly4Ser)3 linker   BamHI

        Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Pro
1543   GGG GGA GGC GGT TCA GGA GGT GGA GGT TCT GGA GGT GGC GGA TCG GAT CCA
        OR ProAspPro linker
          BamHI
        Pro Asp Pro
1543   CCG GAT CCA

        CD154 SHORT FORM Extracellular Domain
1594GS Glu Asn Ser Phe Glu Met Gln Lys
1552PDP GAA AAC AGC TTT GAA ATG CAA AAA
1618GS Gly Asp Gln Asn Pro Gln Ile Ala Ala His Val Ile Ser Glu Ala
1576PDP GGT GAT CAG AAT CCT CAA ATT GCG GCA CAT GTC ATA AGT GAG GCC
1663GS Ser Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu Lys Gly Tyr
1621PDP AGC AGT AAA ACA ACA TCT GTG TTA CAG TGG GCT GAA AAA GGA TAC
1708GS Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly Lys Gln
1666PDP TAC ACC ATG AGC AAC AAC TTG GTA ACC CTG GAA AAT GGG AAA CAG
1753GS Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln Val
1711PDP CTG ACC GTT AAA AGA CAA GGA CTC TAT TAT ATC TAT GCC CAA GTC
1798GS Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe Ile
1756PDP ACC TTC TGT TCC AAT CGG GAA GCT TCG AGT CAA GCT CCA TTT ATA
1843GS Ala Ser Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile Leu
1801PDP GCC AGC CTC TGC CTA AAG TCC CCC GGT AGA TTC GAG AGA ATC TTA
1888GS Leu Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln
1846PDP CTC AGA GCT GCA AAT ACC CAC AGT TCC GCC AAA CCT TGC GGG CAA
1933GS Gln Ser Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala
1891PDP CAA TCC ATT CAC TTG GGA GGA GTA TTT GAA TTG CAA CCA GGT GCT
1978GS Ser Val Phe Val Asn Val Thr Asp Pro Ser Gln Val Ser His Gly
1936PDP TCG GTG TTT GTC AAT GTG ACT GAT CCA AGC CAA GTG AGC CAT GGC
                                                        XbaI
                                                        ~~~
2023GS  Thr Gly Phe Thr Ser Phe Gly Leu Leu Lys Leu Glu *** *** Ser
1981PDP ACT GGC TTC ACG TCC TTT GGC TTA CTC AAA CTC GAG TGA TAA TCT
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SEQUENCE LISTING

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Hayden-Ledbetter, Martha

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Binds CD40

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binds to CD40

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cccacagacc ccaacccaca agaagtagta ttgggaaatg tgacagaaaa ttttaacatg 300

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ataaaaaatt gctctttcta tatcaccaca agcataagaa ataaggtaaa gaaagaatat 540

gcacttttta atagacttga tgtagtacca atagaaaata ctaataatac taagtatagg 600

ttaataagtt gtaacacctc agtcattaca caggcctgtc caaaggtatc ctttcagcca 660

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ttcaatggat caggaccatg cacaaatgtc agcacagtac aatgtacaca tggaattagg 780

ccagtggtgt caactcaact gctgttaaat ggcagtctag cagaagaaga catagtaatt 840

agatctgaaa atttcacaga caatgctaaa accataatag tacagctaaa tgaatctgta 900

gtaattaatt gtacaagacc caacaacaat acaagaagaa ggttatctat aggaccaggg 960

agagcatttt atgcaagaag aaacataata ggagatataa gacaagcaca ttgtaacatt 1020

agtagagcaa aatggaataa cactttacaa cagatagtta taaaattaag agaaaaattt 1080

aggaataaaa caatagcctt taatcaatcc tcaggagggg acccagaaat tgtaatgcac 1140

agttttaatt gtggagggga attcttctac tgtaatacag cacaactgtt taatagtact 1200

tggaatgtta ctggagggac aaatggcact gaaggaaatg acataatcac actccaatgc 1260

agaataaaac aaattataaa tatgtggcag aaagtaggaa aagcaatgta tgcccctccc 1320

atcacaggac aaattagatg ttcatcaaat attacagggc tgctactaac aagagatgga 1380

ggtaatagta ctgagactga gactgagatc ttcagacctg gaggaggaga tatgagggac 1440

aattggagaa gtgaattata taaatataaa gtagtaagaa ttgaaccaat aggagtagca 1500

cccaccaggg caaagagaag aacagtgcaa agagaaaaaa gaggggggagg cggttcagga 1560

ggtggaggtt ctggaggtgg cggatcggat ccagaaaaca gctttgaaat gcaaaaaggt 1620

gatcagaatc ctcaaattgc ggcacatgtc ataagtgagg ccagcagtaa aacaacatct 1680

gtgttacagt gggctgaaaa aggatactac accatgagca acaacttggt aaccctggaa 1740

aatgggaaac agctgaccgt taaaagacaa ggactctatt atatctatgc ccaagtcacc 1800

ttctgttcca atcgggaagc ttcgagtcaa gctccattta tagccagcct ctgcctaaag 1860

tcccccggta gattcgagag aatcttactc agagctgcaa atacccacag ttccgccaaa 1920

ccttgcgggc aacaatccat tcacttggga ggagtatttg aattgcaacc aggtgcttcg 1980

gtgtttgtca atgtgactga tccaagccaa gtgagccatg gcactggctt cacgtccttt 2040

ggcttactca aactcgagtg ataatctaga 2070

<210> 15
<211> 2028

<212> DNA
<213> HIV-HUMAN FUSION CDNA

<220>
<221> sig_peptide
<222> (13)..(72)
<223> synthetic secretory signal peptide

<220>
<221> allele
<222> (73)..(1551)
<223> HIV gp120 + ProAspPro linker

<220>
<221> misc_feature
<222> (1552)..(2028)
<223> CD154 extracellular domain
short form (amino acids 108-261)+Glu
binds CD40

<400> 15
aagcttgccg ccatgctgta tacctctcag ctgttaggac tacttctgtt ttggatctcg 60

gcttcgagat ccatgctcct tgggatattg atgatctgta gtgctacaga aaaattgtgg 120

gtcacagtct attatggggt acctgtgtgg agagaagcaa ccaccactct attttgtgca 180

tcagatgcta aagcctatga tacagaggta cataatgttt gggccacaca tgcctgtgta 240

cccacagacc ccaacccaca agaagtagta ttgggaaatg tgacagaaaa ttttaacatg 300

tggaaaaata acatggtaga tcagatgcat gaggatataa tcagtttatg ggatgaaagc 360

ctaaagccat gtgtaaaatt aaccccactc tgtgttactt taaattgcac taatttgaat 420

atcactaaga atactactaa tcccactagt agcagctggg gaatgatgga gaaaggagaa 480

ataaaaaatt gctctttcta tatcaccaca agcataagaa ataaggtaaa gaaagaatat 540

gcacttttta atagacttga tgtagtacca atagaaaata ctaataatac taagtatagg 600

ttaataagtt gtaacacctc agtcattaca caggcctgtc caaaggtatc ctttcagcca 660

attcccatac attattgtgt cccggctggg tttgcgatgc taaagtgtaa caataagaca 720

ttcaatggat caggaccatg cacaaatgtc agcacagtac aatgtacaca tggaattagg 780

ccagtggtgt caactcaact gctgttaaat ggcagtctag cagaagaaga catagtaatt 840

agatctgaaa atttcacaga caatgctaaa accataatag tacagctaaa tgaatctgta 900

gtaattaatt gtacaagacc caacaacaat acaagaagaa ggttatctat aggaccaggg 960

agagcatttt atgcaagaag aaacataata ggagatataa gacaagcaca ttgtaacatt 1020

agtagagcaa aatggaataa cactttacaa cagatagtta taaaattaag agaaaaattt 1080

aggaataaaa caatagcctt taatcaatcc tcaggagggg acccagaaat tgtaatgcac 1140

agttttaatt gtggagggga attcttctac tgtaatacag cacaactgtt taatagtact 1200

tggaatgtta ctggagggac aaatggcact gaaggaaatg acataatcac actccaatgc 1260

agaataaaac aaattataaa tatgtggcag aaagtaggaa aagcaatgta tgcccctccc 1320

atcacaggac aaattagatg ttcatcaaat attacagggc tgctactaac aagagatgga 1380

ggtaatagta ctgagactga gactgagatc ttcagacctg gaggaggaga tatgagggac 1440

aattggagaa gtgaattata taaatataaa gtagtaagaa ttgaaccaat aggagtagca 1500

cccaccaggg caaagagaag aacagtgcaa agagaaaaaa gaccggatcc agaaaacagc 1560

tttgaaatgc aaaaaggtga tcagaatcct caaattgcgg cacatgtcat aagtgaggcc 1620

agcagtaaaa caacatctgt gttacagtgg gctgaaaaag gatactacac catgagcaac 1680

aacttggtaa ccctggaaaa tgggaaacag ctgaccgtta aaagacaagg actctattat 1740

atctatgccc aagtcacctt ctgttccaat cgggaagctt cgagtcaagc tccatttata 1800

gccagcctct gcctaaagtc ccccggtaga ttcgagagaa tcttactcag agctgcaaat 1860

acccacagtt ccgccaaacc ttgcgggcaa caatccattc acttgggagg agtatttgaa 1920

ttgcaaccag gtgcttcggt gtttgtcaat gtgactgatc caagccaagt gagccatggc 1980

actggcttca cgtcctttgg cttactcaaa ctcgagtgat aatctaga 2028

<210> 16
<211> 906
<212> DNA
<213> HIV-human

<220>
<221> sig_peptide
<222> (13)..(72)
<223> synthetic secretory signal peptide

<220>
<221> misc_structure
<222> (73)..(243)
<223> HIV gp 120 V3 loop with [gly4ser3] linker

<220>
<221> misc_feature
<222> (250)..(906)
<223> human CD154 extracellular domain
long form from amino acids 48-261+Glu
binds CD40

<400> 16
aagcttgccg ccatgctgta tacctctcag ctgttaggac tacttctgtt ttggatctcg 60

gcttcgagat ctgtagtaat taattgtaca agacccaaca acaatacaag aagaaggtta 120

tctataggac cagggagagc attttatgca agaagaaaca taataggaga tataagacaa 180

gcacattgta acattagtgg tggcggtggc tcaggaggcg gtggatctgg cggtggaggt 240

tcggatccaa gaaggttgga caagatagaa gatgaaagga atcttcatga agattttgta 300

ttcatgaaaa cgatacagag atgcaacaca ggagaaagat ccttatcctt actgaactgt 360

gaggagaata aaagccagtt tgaaggcttt gtgaaggata taatgttaaa caaagaggag 420

acgaagaaag aaaacagctt tgaaatgcaa aaaggtgatc agaatcctca aattgcggca 480

catgtcataa gtgaggccag cagtaaaaca acatctgtgt tacagtgggc tgaaaaagga 540

tactacacca tgagcaacaa cttggtaacc ctggaaaatg ggaaacagct gaccgttaaa 600

agacaaggac tctattatat ctatgcccaa gtcaccttct gttccaatcg ggaagcttcg 660

agtcaagctc catttatagc cagcctctgc ctaaagtccc ccggtagatt cgagagaatc 720

ttactcagag ctgcaaatac ccacagttcc gccaaacctt gcgggcaaca atccattcac 780

ttgggaggag tatttgaatt gcaaccaggt gcttcggtgt ttgtcaatgt gactgatcca 840

agccaagtga gccatggcac tggcttcacg tcctttggct tactcaaact cgagtgataa 900

tctaga 906

<210> 17
<211> 865
<212> DNA
<213> HIV-HUMAN FUSION CDNA

<220>
<221> sig_peptide
<222> (13)..(72)
<223> synthetic secretory signal peptide

<220>
<221> misc_feature
<222> (73)..(207)
<223> HIV gp120 V3 loop + ProAspPro linker

<220>
<221> misc_feature
<222> (208)..(865)
<223> CD154 extracellular domain
long form from amino acids 48-261+Glu
binds CD40

<400> 17
aagcttgccg ccatgctgta tacctctcag ctgttaggac tacttctgtt ttggatctcg 60

gcttcgagat ctgtagtaat taattgtaca agacccaaca acaatacaag aagaaggtta 120

tctataggac cagggagagc attttatgca agaagaaaca taataggaga tataagacaa 180

gcacattgta acattagtcc ggatccaaga aggttggaca agatagaaga tgaaaggaat 240

cttcatgaag attttgtatt catgaaaacg atacagagat gcaacacagg agaaagatcc 300

ttatccttac tgaactgtga ggagattaaa agccagtttg aaggctttgt gaaggatata 360

atgttaaaca aagaggagac gaagaaagaa aacagctttg aaatgcaaaa aggtgatcag 420

aatcctcaaa ttgcggcaca tgtcataagt gaggccagca gtaaaacaac atctgtgtta 480

cagtgggctg aaaaaggata ctacaccatg agcaacaact tggtaaccct ggaaaatggg 540

aaacagctga ccgttaaaag acaaggactc tattatatct atgcccaagt caccttctgt 600

tccaatcggg aagcttcgag tcaagctcca tttatagcca gcctctgcct aaagtccccc 660

ggtagattcg agagaatctt actcagagct gcaaataccc acagttccgc caaaccttgc 720

gggcaacaat ccattcactt gggaggagta tttgaattgc aaccaggtgc ttcggtgttt 780

gtcaatgtga ctgatccaag ccaagtgagc catggcactg gcttcacgtc ctttggctta 840

ctcaaactcg agtgataatc tagat 865

<210> 18
<211> 726
<212> DNA
<213> HIV-HUMAN FUSION CDNA

<220>
<221> sig_peptide
<222> (13)..(72)
<223> synthetic secretory signal peptide

<220>
<221> misc_feature
<222> (73)..(207)
<223> HIV gp120 V3 loop plus ProAspPro linker

<220>
<221> misc_feature
<222> (208)..(726)
<223> CD154 extracellular domain
short form from amino acids 108-261+Glu
binds CD40

<400> 18
aagcttgccg ccatgctgta tacctctcag ctgttaggac tacttctgtt ttggatctcg 60

gcttcgagat ctgtagtaat taattgtaca agacccaaca acaatacaag aagaaggtta 120

tctataggac cagggagagc attttatgca agaagaaaca taataggaga tataagacaa 180

gcacattgta acattagtgg tggcggtggc tcaggaggcg gtggatctgg cggtggaggt 240

tcggatccag aaaacagctt tgaaatgcaa aaaggtgatc agaatcctca aattgcggca 300

catgtcataa gtgaggccag cagtaaaaca acatctgtgt tacagtgggc tgaaaaagga 360

tactacacca tgagcaacaa cttggtaacc ctggaaaatg ggaaacagct gaccgttaaa 420

agacaaggac tctattatat ctatgcccaa gtcaccttct gttccaatcg ggaagcttcg 480

agtcaagctc catttatagc cagcctctgc ctaaagtccc ccggtagatt cgagagaatc 540

ttactcagag ctgcaaatac ccacagttcc gccaaacctt gcgggcaaca atccattcac 600

ttgggaggag tatttgaatt gcaaccaggt gcttcggtgt ttgtcaatgt gactgatcca 660

agccaagtga gccatggcac tggcttcacg tcctttggct tactcaaact cgagtgataa 720

tctaga 726

<210> 19
<211> 684
<212> DNA
<213> HIV-human fusion cDNA

<220>
<221> sig_peptide
<222> (13)..(72)
<223> Synthetic secretory signal peptide

<220>
<221> misc_feature
<222> (73)..(207)
<223> HIV gp120 V3 loop with ProAspPro linker

<220>
<221> misc_feature
<222> (208)..(684)
<223> human CD154 extracellular domain
short form from amino acids 108-261+Glu
binds to CD40

<400> 19
aagcttgccg ccatgctgta tacctctcag ctgttaggac tacttctgtt ttggatctcg 60

gcttcgagat ctgtagtaat taattgtaca agacccaaca acaatacaag aagaaggtta 120

tctataggac cagggagagc attttatgca agaagaaaca taataggaga tataagacaa 180

gcacattgta acattagtcc ggatccagaa aacagctttg aaatgcaaaa aggtgatcag 240

aatcctcaaa ttgcggcaca tgtcataagt gaggccagca gtaaaacaac atctgtgtta 300

cagtgggctg aaaaaggata ctacaccatg agcaacaact tggtaaccct ggaaaatggg 360

aaacagctga ccgttaaaag acaaggactc tattatatct atgcccaagt caccttctgt 420

tccaatcggg aagcttcgag tcaagctcca tttatagcca gcctctgcct aaagtccccc 480

ggtagattcg agagaatctt actcagagct gcaaataccc acagttccgc caaaccttgc 540

gggcaacaat ccattcactt gggaggagta tttgaattgc aaccaggtgc ttcggtgttt 600

gtcaatgtga ctgatccaag ccaagtgagc catggcactg gcttcacgtc ctttggctta 660

ctcaaactcg agtgataatc taga 684

<210> 20
<211> 742
<212> PRT
<213> HIV-HUMAN FUSION PROTEIN

<220>
<221> SIGNAL
<222> (1)..(20)
<223> synthetic secretory signal peptide

<220>

<221> DOMAIN
<222> (21)..(526)
<223> HIV gp120 domain with (gly4ser)3 linker

<220>
<221> BINDING
<222> (529)..(742)
<223> CD154 extracellular domain
long form from amino acids 48 (Arg) to 261 (Leu)+Glu

<400> 20

Met Leu Tyr Thr Ser Gln Leu Leu Gly Leu Leu Leu Phe Trp Ile Ser
1 5 10 15

Ala Ser Arg Ser Met Leu Leu Gly Ile Leu Met Ile Cys Ser Ala Thr
20 25 30

Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Arg Glu
35 40 45

Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Asp Thr
50 55 60

Glu Val His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro
65 70 75 80

Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu Asn Phe Asn Met
85 90 95

Trp Lys Asn Asn Met Val Asp Gln Met His Glu Asp Ile Ile Ser Leu
100 105 110

Trp Asp Glu Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val
115 120 125

Thr Leu Asn Cys Thr Asn Leu Asn Ile Thr Lys Asn Thr Thr Asn Pro
130 135 140

Thr Ser Ser Ser Trp Gly Met Met Glu Lys Gly Glu Ile Lys Asn Cys
145 150 155 160

Ser Phe Tyr Ile Thr Thr Ser Ile Arg Asn Lys Val Lys Lys Glu Tyr
165 170 175

Ala Leu Phe Asn Arg Leu Asp Val Val Pro Ile Glu Asn Thr Asn Asn
180 185 190

Thr Lys Tyr Arg Leu Ile Ser Cys Asn Thr Ser Val Ile Thr Gln Ala
195 200 205

Cys Pro Lys Val Ser Phe Gln Pro Ile Pro Ile His Tyr Cys Val Pro
210 215 220

Ala Gly Phe Ala Met Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Ser
225 230 235 240

Gly Pro Cys Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg
245 250 255

Pro Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu
260 265 270

Asp Ile Val Ile Arg Ser Glu Asn Phe Thr Asp Asn Ala Lys Thr Ile
275 280 285

Ile Val Gln Leu Asn Glu Ser Val Val Ile Asn Cys Thr Arg Pro Asn
290 295 300

Asn Asn Thr Arg Arg Arg Leu Ser Ile Gly Pro Gly Arg Ala Phe Tyr
305 310 315 320

Ala Arg Arg Asn Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile
325 330 335

Ser Arg Ala Lys Trp Asn Asn Thr Leu Gln Gln Ile Val Ile Lys Leu
340 345 350

Arg Glu Lys Phe Arg Asn Lys Thr Ile Ala Phe Asn Gln Ser Ser Gly
355 360 365

Gly Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe
370 375 380

Phe Tyr Cys Asn Thr Ala Gln Leu Phe Asn Ser Thr Trp Asn Val Thr
385 390 395 400

Gly Gly Thr Asn Gly Thr Glu Gly Asn Asp Ile Ile Thr Leu Gln Cys
405 410 415

Arg Ile Lys Gln Ile Ile Asn Met Trp Gln Lys Val Gly Lys Ala Met
420 425 430

Tyr Ala Pro Pro Ile Thr Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr
435 440 445

Gly Leu Leu Leu Thr Arg Asp Gly Gly Asn Ser Thr Glu Thr Glu Thr
450 455 460

Glu Ile Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser
465 470 475 480

Glu Leu Tyr Lys Tyr Lys Val Val Arg Ile Glu Pro Ile Gly Val Ala
485 490 495

Pro Thr Arg Ala Lys Arg Arg Thr Val Gln Arg Glu Lys Arg Gly Gly
500 505 510

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Pro Arg
515 520 525

Arg Leu Asp Lys Ile Glu Asp Glu Arg Asn Leu His Glu Asp Phe Val
530 535 540

Phe Met Lys Thr Ile Gln Arg Cys Asn Thr Gly Glu Arg Ser Leu Ser
545 550 555 560

Leu Leu Asn Cys Glu Glu Ile Lys Ser Gln Phe Glu Gly Phe Val Lys
565 570 575

Asp Ile Met Leu Asn Lys Glu Glu Thr Lys Lys Glu Asn Ser Phe Glu
580 585 590

Met Gln Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala His Val Ile Ser
595 600 605

Glu Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu Lys Gly
610 615 620

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Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly Lys Gln
625                 630                 635                 640

Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln Val Thr
                645                 650                 655

Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe Ile Ala Ser
            660                 665                 670

Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile Leu Leu Arg Ala
        675                 680                 685

Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln Gln Ser Ile His
    690                 695                 700

Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser Val Phe Val Asn
705                 710                 715                 720

Val Thr Asp Pro Ser Gln Val Ser His Gly Thr Gly Phe Thr Ser Phe
                725                 730                 735

Gly Leu Leu Lys Leu Glu
            740
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<210> 21
<211> 728
<212> PRT
<213> HIV-HUMAN FUSION PROTEIN

<220>
<221> SIGNAL
<222> (1)..(20)
<223> Synthetic secretory signal peptide

<220>
<221> DOMAIN
<222> (21)..(513)
<223> HIV gp120 domain plus ProAspPro linker

<220>
<221> BINDING
<222> (514)..(728)
<223> CD154 extracellular domain
long form from amino acids 48 (Arg) to 261 (Leu)+Glu
Binds CD40

<400> 21

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Met Leu Tyr Thr Ser Gln Leu Leu Gly Leu Leu Leu Phe Trp Ile Ser
1               5                   10                  15

Ala Ser Arg Ser Met Leu Leu Gly Ile Leu Met Ile Cys Ser Ala Thr
            20                  25                  30

Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Arg Glu
        35                  40                  45

Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Asp Thr
    50                  55                  60

Glu Val His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro
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65 70 75 80

Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu Asn Phe Asn Met
85 90 95

Trp Lys Asn Asn Met Val Asp Gln Met His Glu Asp Ile Ile Ser Leu
100 105 110

Trp Asp Glu Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val
115 120 125

Thr Leu Asn Cys Thr Asn Leu Asn Ile Thr Lys Asn Thr Thr Asn Pro
130 135 140

Thr Ser Ser Ser Trp Gly Met Met Glu Lys Gly Glu Ile Lys Asn Cys
145 150 155 160

Ser Phe Tyr Ile Thr Thr Ser Ile Arg Asn Lys Val Lys Lys Glu Tyr
165 170 175

Ala Leu Phe Asn Arg Leu Asp Val Val Pro Ile Glu Asn Thr Asn Asn
180 185 190

Thr Lys Tyr Arg Leu Ile Ser Cys Asn Thr Ser Val Ile Thr Gln Ala
195 200 205

Cys Pro Lys Val Ser Phe Gln Pro Ile Pro Ile His Tyr Cys Val Pro
210 215 220

Ala Gly Phe Ala Met Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Ser
225 230 235 240

Gly Pro Cys Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg
245 250 255

Pro Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu
260 265 270

Asp Ile Val Ile Arg Ser Glu Asn Phe Thr Asp Asn Ala Lys Thr Ile
275 280 285

Ile Val Gln Leu Asn Glu Ser Val Val Ile Asn Cys Thr Arg Pro Asn
290 295 300

Asn Asn Thr Arg Arg Arg Leu Ser Ile Gly Pro Gly Arg Ala Phe Tyr
305 310 315 320

Ala Arg Arg Asn Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile
325 330 335

Ser Arg Ala Lys Trp Asn Asn Thr Leu Gln Gln Ile Val Ile Lys Leu
340 345 350

Arg Glu Lys Phe Arg Asn Lys Thr Ile Ala Phe Asn Gln Ser Ser Gly
355 360 365

Gly Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe
370 375 380

Phe Tyr Cys Asn Thr Ala Gln Leu Phe Asn Ser Thr Trp Asn Val Thr
385 390 395 400

Gly Gly Thr Asn Gly Thr Glu Gly Asn Asp Ile Ile Thr Leu Gln Cys
405 410 415

Arg Ile Lys Gln Ile Ile Asn Met Trp Gln Lys Val Gly Lys Ala Met
420 425 430

Tyr Ala Pro Pro Ile Thr Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr
435 440 445

Gly Leu Leu Leu Thr Arg Asp Gly Gly Asn Ser Thr Glu Thr Glu Thr
450 455 460

Glu Ile Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser
465 470 475 480

Glu Leu Tyr Lys Tyr Lys Val Val Arg Ile Glu Pro Ile Gly Val Ala
485 490 495

Pro Thr Arg Ala Lys Arg Arg Thr Val Gln Arg Glu Lys Arg Pro Asp
500 505 510

Pro Arg Arg Leu Asp Lys Ile Glu Asp Glu Arg Asn Leu His Glu Asp
515 520 525

Phe Val Phe Met Lys Thr Ile Gln Arg Cys Asn Thr Gly Glu Arg Ser
530 535 540

Leu Ser Leu Leu Asn Cys Glu Glu Ile Lys Ser Gln Phe Glu Gly Phe
545 550 555 560

Val Lys Asp Ile Met Leu Asn Lys Glu Glu Thr Lys Lys Glu Asn Ser
565 570 575

Phe Glu Met Gln Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala His Val
580 585 590

Ile Ser Glu Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu
595 600 605

Lys Gly Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly
610 615 620

Lys Gln Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln
625 630 635 640

Val Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe Ile
645 650 655

Ala Ser Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile Leu Leu
660 665 670

Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln Gln Ser
675 680 685

Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser Val Phe
690 695 700

Val Asn Val Thr Asp Pro Ser Gln Val Ser His Gly Thr Gly Phe Thr
705 710 715 720

Ser Phe Gly Leu Leu Lys Leu Glu
725

<210> 22
<211> 682
<212> PRT
<213> HIV-HUMAN FUSION PROTEIN

<220>
<221> SIGNAL
<222> (1)..(20)
<223> Synthetic secretory signal peptide

<220>
<221> DOMAIN
<222> (21)..(525)
<223> HIV gp120 domain plus (gly4ser)3 linker

<220>
<221> DOMAIN
<222> (528)..(682)
<223> CD154 extracellular domain
short form from amino acids 108 (Glu) to 261 (Leu)+Glu
Binds CD40

<400> 22

Met Leu Tyr Thr Ser Gln Leu Leu Gly Leu Leu Leu Phe Trp Ile Ser
1 5 10 15

Ala Ser Arg Ser Met Leu Leu Gly Ile Leu Met Ile Cys Ser Ala Thr
20 25 30

Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Arg Glu
35 40 45

Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Asp Thr
50 55 60

Glu Val His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro
65 70 75 80

Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu Asn Phe Asn Met
85 90 95

Trp Lys Asn Asn Met Val Asp Gln Met His Glu Asp Ile Ile Ser Leu
100 105 110

Trp Asp Glu Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val
115 120 125

Thr Leu Asn Cys Thr Asn Leu Asn Ile Thr Lys Asn Thr Thr Asn Pro
130 135 140

Thr Ser Ser Ser Trp Gly Met Met Glu Lys Gly Glu Ile Lys Asn Cys
145 150 155 160

Ser Phe Tyr Ile Thr Thr Ser Ile Arg Asn Lys Val Lys Lys Glu Tyr
165 170 175

Ala Leu Phe Asn Arg Leu Asp Val Val Pro Ile Glu Asn Thr Asn Asn
180 185 190

Thr Lys Tyr Arg Leu Ile Ser Cys Asn Thr Ser Val Ile Thr Gln Ala
195 200 205

Cys Pro Lys Val Ser Phe Gln Pro Ile Pro Ile His Tyr Cys Val Pro
210 215 220

Ala Gly Phe Ala Met Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Ser

225 230 235 240

Gly Pro Cys Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg
245 250 255

Pro Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu
260 265 270

Asp Ile Val Ile Arg Ser Glu Asn Phe Thr Asp Asn Ala Lys Thr Ile
275 280 285

Ile Val Gln Leu Asn Glu Ser Val Val Ile Asn Cys Thr Arg Pro Asn
290 295 300

Asn Asn Thr Arg Arg Arg Leu Ser Ile Gly Pro Gly Arg Ala Phe Tyr
305 310 315 320

Ala Arg Arg Asn Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile
325 330 335

Ser Arg Ala Lys Trp Asn Asn Thr Leu Gln Gln Ile Val Ile Lys Leu
340 345 350

Arg Glu Lys Phe Arg Asn Lys Thr Ile Ala Phe Asn Gln Ser Ser Gly
355 360 365

Gly Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe
370 375 380

Phe Tyr Cys Asn Thr Ala Gln Leu Phe Asn Ser Thr Trp Asn Val Thr
385 390 395 400

Gly Gly Thr Asn Gly Thr Glu Gly Asn Asp Ile Ile Thr Leu Gln Cys
405 410 415

Arg Ile Lys Gln Ile Ile Asn Met Trp Gln Lys Val Gly Lys Ala Met
420 425 430

Tyr Ala Pro Pro Ile Thr Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr
435 440 445

Gly Leu Leu Leu Thr Arg Asp Gly Gly Asn Ser Thr Glu Thr Glu Thr
450 455 460

Glu Ile Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser
465 470 475 480

Glu Leu Tyr Lys Tyr Lys Val Val Arg Ile Glu Pro Ile Gly Val Ala
485 490 495

Pro Thr Arg Ala Lys Arg Arg Thr Val Gln Arg Glu Lys Arg Gly Gly
500 505 510

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Pro Glu
515 520 525

Asn Ser Phe Glu Met Gln Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala
530 535 540

His Val Ile Ser Glu Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp
545 550 555 560

Ala Glu Lys Gly Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu
565 570 575

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Asn Gly Lys Gln Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr
            580                 585                 590

Ala Gln Val Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro
        595                 600                 605

Phe Ile Ala Ser Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile
    610                 615                 620

Leu Leu Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln
625                 630                 635                 640

Gln Ser Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser
                645                 650                 655

Val Phe Val Asn Val Thr Asp Pro Ser Gln Val Ser His Gly Thr Gly
            660                 665                 670

Phe Thr Ser Phe Gly Leu Leu Lys Leu Glu
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<210> 23
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<213> HIV-HUMAN FUSION PROTEIN

<220>
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<222> (1)..(20)
<223> Synthetic secretory signal peptide

<220>
<221> DOMAIN
<222> (21)..(513)
<223> HIV gp120 domain with ProAspPro linker

<220>
<221> BINDING
<222> (514)..(668)
<223> CD154 extracellular domain
short form from amino acids 108 (Glu) to 261 (Leu)+Glu
Binds to CD40

<400> 23

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1               5                   10                  15

Ala Ser Arg Ser Met Leu Leu Gly Ile Leu Met Ile Cys Ser Ala Thr
            20                  25                  30

Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Arg Glu
        35                  40                  45

Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Asp Thr
    50                  55                  60

Glu Val His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro
65                  70                  75                  80

Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu Asn Phe Asn Met
                85                  90                  95
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Trp Lys Asn Asn Met Val Asp Gln Met His Glu Asp Ile Ile Ser Leu
100 105 110

Trp Asp Glu Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val
115 120 125

Thr Leu Asn Cys Thr Asn Leu Asn Ile Thr Lys Asn Thr Thr Asn Pro
130 135 140

Thr Ser Ser Ser Trp Gly Met Met Glu Lys Gly Glu Ile Lys Asn Cys
145 150 155 160

Ser Phe Tyr Ile Thr Thr Ser Ile Arg Asn Lys Val Lys Lys Glu Tyr
165 170 175

Ala Leu Phe Asn Arg Leu Asp Val Val Pro Ile Glu Asn Thr Asn Asn
180 185 190

Thr Lys Tyr Arg Leu Ile Ser Cys Asn Thr Ser Val Ile Thr Gln Ala
195 200 205

Cys Pro Lys Val Ser Phe Gln Pro Ile Pro Ile His Tyr Cys Val Pro
210 215 220

Ala Gly Phe Ala Met Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Ser
225 230 235 240

Gly Pro Cys Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg
245 250 255

Pro Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu
260 265 270

Asp Ile Val Ile Arg Ser Glu Asn Phe Thr Asp Asn Ala Lys Thr Ile
275 280 285

Ile Val Gln Leu Asn Glu Ser Val Val Ile Asn Cys Thr Arg Pro Asn
290 295 300

Asn Asn Thr Arg Arg Arg Leu Ser Ile Gly Pro Gly Arg Ala Phe Tyr
305 310 315 320

Ala Arg Arg Asn Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile
325 330 335

Ser Arg Ala Lys Trp Asn Asn Thr Leu Gln Gln Ile Val Ile Lys Leu
340 345 350

Arg Glu Lys Phe Arg Asn Lys Thr Ile Ala Phe Asn Gln Ser Ser Gly
355 360 365

Gly Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe
370 375 380

Phe Tyr Cys Asn Thr Ala Gln Leu Phe Asn Ser Thr Trp Asn Val Thr
385 390 395 400

Gly Gly Thr Asn Gly Thr Glu Gly Asn Asp Ile Ile Thr Leu Gln Cys
405 410 415

Arg Ile Lys Gln Ile Ile Asn Met Trp Gln Lys Val Gly Lys Ala Met
420 425 430

Tyr Ala Pro Pro Ile Thr Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr

435 440 445

Gly Leu Leu Leu Thr Arg Asp Gly Gly Asn Ser Thr Glu Thr Glu Thr
450 455 460

Glu Ile Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser
465 470 475 480

Glu Leu Tyr Lys Tyr Lys Val Val Arg Ile Glu Pro Ile Gly Val Ala
485 490 495

Pro Thr Arg Ala Lys Arg Arg Thr Val Gln Arg Glu Lys Arg Pro Asp
500 505 510

Pro Glu Asn Ser Phe Glu Met Gln Lys Gly Asp Gln Asn Pro Gln Ile
515 520 525

Ala Ala His Val Ile Ser Glu Ala Ser Ser Lys Thr Thr Ser Val Leu
530 535 540

Gln Trp Ala Glu Lys Gly Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr
545 550 555 560

Leu Glu Asn Gly Lys Gln Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr
565 570 575

Ile Tyr Ala Gln Val Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln
580 585 590

Ala Pro Phe Ile Ala Ser Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu
595 600 605

Arg Ile Leu Leu Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys
610 615 620

Gly Gln Gln Ser Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro Gly
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Ala Ser Val Phe Val Asn Val Thr Asp Pro Ser Gln Val Ser His Gly
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Thr Gly Phe Thr Ser Phe Gly Leu Leu Lys Leu Glu
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<223> Synthetic secretory signal peptide

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<222> (21)..(77)
<223> HIV gp120 V3 loop plus (gly4ser)3 linker

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<221> BINDING
<222> (80)..(294)
<223> CD154 extracellular domain

long form from amino acids 48 (Arg) to 261 (Leu)+Glu binds CD40

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Met Leu Tyr Thr Ser Gln Leu Leu Gly Leu Leu Leu Phe Trp Ile Ser
1 5 10 15

Ala Ser Arg Ser Val Val Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
20 25 30

Arg Arg Arg Leu Ser Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg
35 40 45

Asn Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Gly Gly
50 55 60

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Pro Arg
65 70 75 80

Arg Leu Asp Lys Ile Glu Asp Glu Arg Asn Leu His Glu Asp Phe Val
85 90 95

Phe Met Lys Thr Ile Gln Arg Cys Asn Thr Gly Glu Arg Ser Leu Ser
100 105 110

Leu Leu Asn Cys Glu Glu Ile Lys Ser Gln Phe Glu Gly Phe Val Lys
115 120 125

Asp Ile Met Leu Asn Lys Glu Glu Thr Lys Lys Glu Asn Ser Phe Glu
130 135 140

Met Gln Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala His Val Ile Ser
145 150 155 160

Glu Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu Lys Gly
165 170 175

Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly Lys Gln
180 185 190

Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln Val Thr
195 200 205

Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe Ile Ala Ser
210 215 220

Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile Leu Leu Arg Ala
225 230 235 240

Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln Gln Ser Ile His
245 250 255

Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser Val Phe Val Asn
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Gly Leu Leu Lys Leu Glu
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<220>
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long form from amino acids 48 (Arg) to 261 (Leu)+Glu
binds CD40

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Met Leu Tyr Thr Ser Gln Leu Leu Gly Leu Leu Leu Phe Trp Ile Ser
1 5 10 15

Ala Ser Arg Ser Val Val Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
20 25 30

Arg Arg Arg Leu Ser Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg
35 40 45

Asn Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Pro Asp
50 55 60

Pro Arg Arg Leu Asp Lys Ile Glu Asp Glu Arg Asn Leu His Glu Asp
65 70 75 80

Phe Val Phe Met Lys Thr Ile Gln Arg Cys Asn Thr Gly Glu Arg Ser
85 90 95

Leu Ser Leu Leu Asn Cys Glu Glu Ile Lys Ser Gln Phe Glu Gly Phe
100 105 110

Val Lys Asp Ile Met Leu Asn Lys Glu Glu Thr Lys Lys Glu Asn Ser
115 120 125

Phe Glu Met Gln Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala His Val
130 135 140

Ile Ser Glu Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu
145 150 155 160

Lys Gly Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly
165 170 175

Lys Gln Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln
180 185 190

Val Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe Ile
195 200 205

Ala Ser Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile Leu Leu

210 215 220

Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln Gln Ser
225 230 235 240

Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser Val Phe
245 250 255

Val Asn Val Thr Asp Pro Ser Gln Val Ser His Gly Thr Gly Phe Thr
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Ser Phe Gly Leu Leu Lys Leu Glu
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short form from amino acids 108 (Glu) to 261 (Leu)+Glu
binds CD40

<400> 26

Met Leu Tyr Thr Ser Gln Leu Leu Gly Leu Leu Leu Phe Trp Ile Ser
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Ala Ser Arg Ser Val Val Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
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Arg Arg Arg Leu Ser Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg
35 40 45

Asn Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Gly Gly
50 55 60

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Pro Glu
65 70 75 80

Asn Ser Phe Glu Met Gln Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala
85 90 95

His Val Ile Ser Glu Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp
100 105 110

Ala Glu Lys Gly Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu
115 120 125

Asn Gly Lys Gln Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr
130 135 140

Ala Gln Val Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro
145 150 155 160

Phe Ile Ala Ser Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile
165 170 175

Leu Leu Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln
180 185 190

Gln Ser Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser
195 200 205

Val Phe Val Asn Val Thr Asp Pro Ser Gln Val Ser His Gly Thr Gly
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Phe Thr Ser Phe Gly Leu Leu Lys Leu Glu
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<222> (66)..(220)
<223> CD154 extracellular domain from amino acids 108 (Glu)-261(Leu)+Glu
Binds CD40

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Met Leu Tyr Thr Ser Gln Leu Leu Gly Leu Leu Leu Phe Trp Ile Ser
1 5 10 15

Ala Ser Arg Ser Val Val Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
20 25 30

Arg Arg Arg Leu Ser Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg
35 40 45

Asn Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Pro Asp
50 55 60

Pro Glu Asn Ser Phe Glu Met Gln Lys Gly Asp Gln Asn Pro Gln Ile
65 70 75 80

Ala Ala His Val Ile Ser Glu Ala Ser Ser Lys Thr Thr Ser Val Leu
85 90 95

Gln Trp Ala Glu Lys Gly Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr
100 105 110

Leu Glu Asn Gly Lys Gln Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr
115 120 125

Ile Tyr Ala Gln Val Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln
130 135 140

Ala Pro Phe Ile Ala Ser Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu
145 150 155 160

Arg Ile Leu Leu Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys
165 170 175

Gly Gln Gln Ser Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro Gly
180 185 190

Ala Ser Val Phe Val Asn Val Thr Asp Pro Ser Gln Val Ser His Gly
195 200 205

Thr Gly Phe Thr Ser Phe Gly Leu Leu Lys Leu Glu
210 215 220

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(21) International Application Number: PCT/US00/28414

(22) International Filing Date: 13 October 2000 (13.10.2000)

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(30) Priority Data:
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(72) Inventors: **LEDBETTER, Jeffrey, A.** [US/US]; 18798 Ridgefield Road N.W., Shoreline, WA 98177-3227 (US). **HAYDEN-LEDBETTER, Martha, S.** [US/US]; 18798 Ridgefield Road N.W., Shoreline, WA 98177-3227 (US).

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(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
18 October 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/26608 A3

(54) Title: DNA VACCINES ENCODING ANTIGEN LINKED TO A DOMAIN THAT BINDS CD40

(57) Abstract: Vaccines that target one or more antigens to a cell surface receptor improve the antigen-specific humoral and cellular immune response. Antigen(s) linked to a domain that binds to a cell surface receptor are internalized, carrying antigen(s) into an intracellular compartment where the antigen(s) are digested into peptides and loaded onto MHC molecules. T cells specific for the peptide antigens are activated, leading to an enhanced immune response. The vaccine may comprise antigen(s) linked to a domain that binds at least one receptor or a DNA plasmid encoding antigen(s) linked to a domain that binds at least one receptor. A preferred embodiment of the invention targets HIV-1 env antigen to the CD40 receptor, resulting in delivery of antigen to CD40 positive cells, and selective activation of the CD40 receptor on cells presenting HIV-1 env antigens to T cells.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/28414

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 39/00; C12P 21/06, 21/04; C12N 15/00

US CL : 424/184.1, 192.1; 435/69.1, 69.7, 320.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/184.1, 192.1; 435/69.1, 69.7, 320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

USPATFUL, WPIDS, MEDLINE, AIDSLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,580,773 A (KANG C.-Y. and L. LUO) 03 December 1996, see entire document.	1, 6, 8, 13
X	US 5,945,513 A (ARUFFO A., et al.) 31 August 1999, see entire document.	1, 2, 8, 9
X	US 5,521,288 A (LINSLEY, P. S., et al.) 28 May 1996, see entire document.	1, 8
X — Y	US 5,698,679 A (NEMAZEE, D. A.) 16 December 1997, see entire document.	1, 8 — 2, 4, 5, 9, 11, 12

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

15 DECEMBER 2000

Date of mailing of the international search report

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Name and mailing address of the ISA/US
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